NEW COUPLING AGENTS FOR PEPTIDE SYNTHESIS

GOVERNMENT SUPPORT

[0001]   This work has been supported by a grant form the National Institutes of Health GM-09706 and the National Science Foundation (CHE-9003192). The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002]   The present invention relates to a new process for effecting the acylation step in amide formation, especially in peptide synthesis.

Description of the Prior Art

[0003]   Polypeptides, especially proteins, play a critical role in fundamental biochemical processes in living cells. Biochemical reactions, including metabolic reactions, are catalyzed by enzymes, which are comprised of proteins. These proteins are chiral molecules, and it is often the case that of the various stereoisomers that may possibly exist, one is usually the most efficacious.

[0004]   Moreover, polypeptides are useful as medicaments. In recent years, peptides have been found useful in combating various diseases, including cancer, diabetes, plant toxins, and the like. Additionally, peptides have shown specific activity as growth promoters, suppressants, antibiotics, insecticides, contraceptives, anti-hypertensives, sleep inducers, anti-depressants, analgesics, and so on.

[0005]   The synthesis of proteins has always been a challenge to chemists. However, chemical synthesis offers advantages not realized by genetic engineering and other biological approaches such as isolation of natural proteins. First, it is useful in confirming the structure of a protein. Moreover, protein

synthesis is necessary to synthesize analogs, allowing scientists to evaluate biological activity and/or pharmacological efficacy in relation to molecular structure.

[0006]   Success in the chemical synthesis of peptides relies, in part, on the use of the appropriate coupling reagents in combination with the appropriate protecting groups. Especially in peptide synthesis, formation of the peptide bond between two amino acids requires activation of the carboxyl group of one of the amino acids before the reaction can occur. However, the activation step in conjunction with the coupling reaction causes a serious problem of loss of configuration at the carboxyl residue which has been activated. Thus, in designing chemical syntheses of peptides, the objective is to provide the peptide product in good yield and maintenance of the configurational integrity of the carboxylic component, i.e., minimal racemization. Thus, the duality of good yield and minimal or no racemization is difficult to achieve because the best methods require the acid to be converted to a derivative bearing a good leaving group. Thus, under normal coupling conditions, there is a loss of configuration.

[0007]   Moreover, current methods of syntheses also tend to produce side reactions which decrease yield.

[0008]   Currently, syntheses of peptides are in solution by classical or various repetitive methods. Alternatively, peptides may be prepared on a solid support (Merrifield method). These are all popular techniques in synthesizing peptides from the coupling of two or more amino acids, in synthesizing larger peptides from the coupling of amino acids with smaller peptides or in the coupling of smaller peptides. Solution methods have the advantage of being easily monitored, allowing purification of intermediates, if necessary, at any stage. A major drawback, however, is the relative slow pace of synthesis, with each step being carried out manually.

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[0009]   The major advantage of the Merrifield method is its easy automation so that unattended, computer-controlled machine synthesis is possible. Unfortunately, the method suffers from an inherent deficiency due to the insoluble nature of the support on which the synthesis proceeds. Unless each acylation step occurs with approximately 100% efficiency, mixtures will inevitably be built up on the polymer. The longer the chain, the greater will be the contamination by undesired side reactions. Side products produced in such reactions remain to contaminate the desired product when it is removed from the polymeric matrix at the end of the cycle. These current techniques are not useful in preparing peptides of greater than 40-50 residues; separation of side products from the desired product becomes increasingly difficult when larger peptides are synthesized.

[0010]   For very long segments (50 or more amino acids), therefore, current methods are not satisfactory. Often, mixtures are obtained of such forbidding complexity that it may be difficult or impossible to isolate the desired peptide.

[0011]   The problems enumerated hereinabove may be eliminated if the proper derivatives of the underlying amino acids and/or the proper conditions for the coupling reaction could be found. Protecting groups, such as t-butyloxy-carbonyl (t-Boc) or N-α-(9-fluorenylmethyl)oxycarbonyl (Fmoc), have been used to minimize side reactions.

[0012]   The most commonly used coupling reagents are carbodiimides such as dicyclohexylcarbodiimides, diisopropylcarbodiimides, 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimides used with various additives.

[0013]   Additives generally inhibit side reactions and reduce racemization. Heretofore, the most common peptide coupling additive used during peptide coupling for peptide synthesis is 1-hydroxybenzotriazole (HOBt). This reagent

has been used either in combination with a carbodiimide or other coupling agent or built into a stand alone reagent, such as 1-benzotriazolyoxytris (dimethylamino) phosphonium hexafluorophosphate (BOP) or an analogous uronium salt. HOBt is applicable to both stepwise and segment condensations. However, many cases have been encountered in which HOBt is ineffective, possibly because of steric effects, or low basicity of the amino component. Especially problematic are segment couplings at amino acid units other than glycine or proline, since the problem of racemization may be severe. The related N-hydroxybenzotriazinone (HOOBt) may provide better protection against racemization, but it is rarely used due to competing side reactions involving ring openings. A drawback in the use of BOP is that it produces a toxic side product, hexamethylphosphorotriamide.
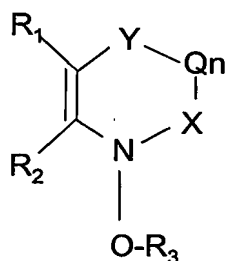
[0014]   Recently other coupling reagents have been introduced, such as N-[1-H-benzotriazo-1-yl)(dimethylamino)methylene]-N-methylmethan-aminiumhexafluorophosphate N-oxide (HBTU), N-[(1-H-benzotriazol)dimethylamino) methylene] N-methylmethanaminium tetrafluoroborate N-oxide (TBTU), O-(benzotriazol-1-yl)-1,3-dimethyl-1,3-dimethylene uranium hexafluorophosphate (HBMDU), O-(benzotriazol-1-yl)-1, 1, 3, 3-bis(tetramethylene)uronium hexafluorophosphate (HBPyU) and O-(benzotriazol-1-yl)- 1, 1, 3, 3-bis(pentamethylene)uronium hexafluorophosphate (HBPipU).

[0015]   Another additive that has been used in peptide synthesis is 3-hydroxy-4-oxo-3, 4-dihydro-1, 2, 3-benzotriazine (HODhbt). HODhbt has proved to be generally superior to HOBt. Moreover, its use permits one to follow the completion of the reaction visually by a color change which occurs when acylation is complete. However, HODhbt has problems associated therewith due to inherent side reactions.

[0016]   Other derivatives, which include O-(3, 4-dihydro-4-oxo-1, 2, 3-
benzotriazin-3-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate, O-(3,4-
dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1, 1, 3, 1-tetra-methyluronium
tetrafluoroborate and [3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-
yl)oxy]tris(pyrrolidino)phosphonium hexafluorophosphate also have applications
in peptide coupling.

[0017]   Other reagents for facilitating peptide coupling have also been
described. For example, in Tetrahedron Letters, 1994, 2279-2282, Carpino, et al.
disclose that 1-hydroxy-7-azabenzotriazole and its corresponding uronium and
phophonium salts, designated HAPyU and AOP, respectively, were effective in
promoting peptide coupling and avoiding racemization in a model solid-phase
peptide segment coupling process. In addition, Carpino, et al. disclose in J. Org.
Chem., 1994, 59, 695-698 that azabenzotriazolyluronium salts, e.g., designated as
HBTU, HATU, HAPyU, and HAMDU, are useful in peptide synthesis.

[0018]   U.S. Patent No. 5,644,029 to Carpino discloses, among other
things, the use of compounds of the following formula in promoting peptide
coupling:

$$\begin{array}{c}
R_1 \\
\diagup \quad Y \\
\quad \diagdown Q_n \\
\quad \quad \mid \\
\diagup \quad N \diagdown X \\
R_2 \quad \mid \\
\quad O\text{-}R_3
\end{array}$$

or N-oxides thereof or salts thereof wherein

R$_1$ and R$_2$ taken together with the carbon atoms to which they are
attached form a heteroaryl ring wherein said heteroaryl ring is an oxygen, sulfur
or nitrogen containing heteroaromatic containing from 3 and up to a total of 13

ring carbon atoms, said heteroaryl may be unsubstituted or substituted with lower alkyl or an electron-donating group;

Y is O, NR_4, CR_4R_5;

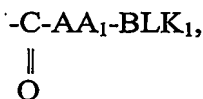R_5 is independently hydrogen or lower alkyl;

X is CR_6R_7 or NR_6;

R_6 and R_7 are independently hydrogen or lower alkyl; or R_6 and R_7 taken together form an oxo group or when n =0, R_4 and R_6 taken together may form a bond between the nitrogen or carbon atom of Y and the nitrogen or carbon atom of X;
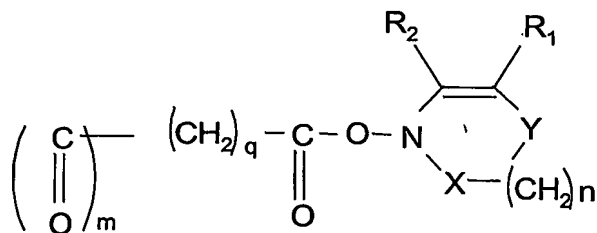
Q is (CR_8R_9) or (NR_8);

when n is 1, R_4 and R_8 taken together may form a bond between the ring carbon or nitrogen atom of Q and the ring carbon or nitrogen atom of R_8;

n is O or 1;

R_3 is hydrogen, lower alkyl carbonyl, aryl carbonyl, lower aryl alkyl carbonyl,

$$\underset{\underset{O}{\parallel}}{\text{-C}}\text{-AA}_1\text{-BLK}_1,$$

a positively charged electron withdrawing group, SO_2R_{14}, or

$$\left(\underset{\underset{O}{\parallel}}{C}\right)_m \quad (CH_2)_q - \underset{\underset{O}{\parallel}}{C} - O - N \overset{\displaystyle R_2 \diagup \diagdown R_1}{\underset{X-(CH_2)_n}{\diagdown Y}}$$

R_{14} is lower alkyl, aryl or lower arylalkyl; q is 0-3;

R_8 and R_9 are independently hydrogen or lower alkyl or R_7 and R_8 taken together with the carbon to which they are attached form an aryl ring, AA_1 is an amino acid and BLK is an amino protecting group, and m is 0 or 1.

[0019]   The present inventor has found other coupling agents, which provide relatively pure products with little, if any, side products being co-produced and minimal, if any, racemization. Moreover, the reaction conditions are very mild and the reagents used are easy to prepare. Thus, by using the compounds of the present invention as additives, the yield of the peptide s enhanced and little, if any, racemization occurs.

SUMMARY OF THE INVENTION

[0020]   The present invention relates to a compound of Formula I and the use thereof in the preparation of a peptide bond in peptide synthesis, said compound having the formula:

I
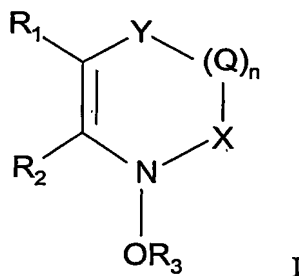
            or N-oxide thereof or salts of said compounds of Formula I wherein

            R_1 and R_2 taken together with the carbon atom to which they are attached form an aryl or a heteroaryl ring, wherein said aryl ring is an aromatic ring containing 6-14 ring carbon atoms and heteroaryl ring is an oxygen, sulfur, or nitrogen containing heteroaromatic containing at least 1 and up to 4 ring heteroatoms selected form oxygen, nitrogen and sulfur and containing from 3 and up to a total of 13 ring carbon atoms, said aryl and heteroaryl may each independently be unsubstituted or substituted with lower alkyl or electron donating group or electron withdrawing group;

            Q is CR_8R_9 or NR_8;

            Y is O, NR_4 or CR_4R_5;

            X is CR_6R_7 or NR_6;

            R_5 is hydrogen or lower alkyl;

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R$_4$ is hydrogen or lower alkyl or

R$_4$ and R$_6$ may form a bond between X and Y, when Y is NR$_4$ or CR$_4$R$_5$ and when Q is not present, or R$_4$ and R$_8$ may form a bond between Y and Q when Y is NR$_4$ or CR$_4$R$_5$ and Q is present, or R$_6$ and R$_8$ may form a bond between Q and X when Q is present;

R$_8$ and R$_9$ are independently hydrogen or lower alkyl, or when Q is present, R$_8$ taken together with R$_4$ may form a bond between Q and Y, when Y is NR$_4$ or CR$_4$R$_5$ or when Q is present, R$_8$ and R$_6$ may form a bond between Q and X;

R$_6$ and R$_7$ are independently hydrogen or lower alkyl or R$_6$ and R$_7$ taken together form an oxo; or R$_6$, taken together with R$_4$, may form a bond between Y and X when Q is not present and Y is NR$_4$ or CR$_4$R$_5$ or R$_6$ and R$_8$ taken together may form a bond between Q and X when Q is present; but in no circumstances is there a double bond between X and Q and Q and Y at the same time;

n is O or 1;
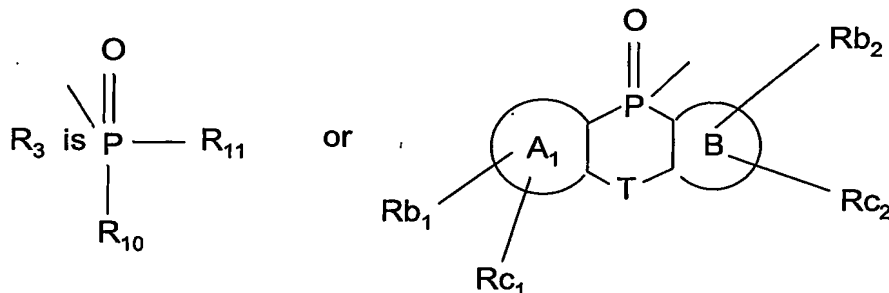
R$_3$ is

R$_{10}$ is OR$_{12}$, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, cycloalkenyl, or cycloalkenyl lower alkyl;

R$_{11}$ is OR$_{13}$, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic or heterocyclic lower alkyl cycloalkenyl or cycloalkenyl lower alkyl or R$_{10}$ and R$_{11}$ may be connected by a

bridging group consisting of S, O, NR_{30} or $(CHR_{30})_m$, wherein R_{30} is lower alkyl or H and m is 1-3 such that R_{10} and R_{11} taken together with the phosphorous atoms form a 5 or 6 membered ring;

R_{12} and R_{13} are independently lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, cycloalkenyl, or cycloalkenyl lower alkyl;

Rings A_1 and B are independently aromatic rings containing 6 to 14 ring carbon atoms or cycloalkenyl or cycloalkyl each containing 5 to 14 ring carbon atoms;

T is CHR_{31}, O, S, or, NR_{31};

R_{31} is lower alkyl or H; and

Rb_1, Rb_2, Rc_1 and Rc_2 are independently hydrogen, lower alkyl or an electron donating group.

[0021]   In another embodiment, the present invention is directed to a compound of formula II or to a salt, in which the cationic portion has the structure of Formula II and to the use of the compound or salt:

II
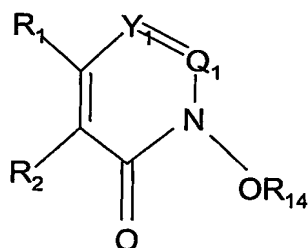
or N-oxides thereof or salts of said compound of Formula II wherein

R_1 and R_2 taken together with the carbon atom to which they are attached form a heteroaryl ring, wherein said heteroaryl is an heteroaromatic containing at least 1 and up to 4 ring heteroatoms selected from O, S and N and containing from 3 and up to a total of 13 ring carbon atoms, said heteroaryl may be unsubstituted or substituted with lower alkyl or electron donating group or electron withdrawing group;
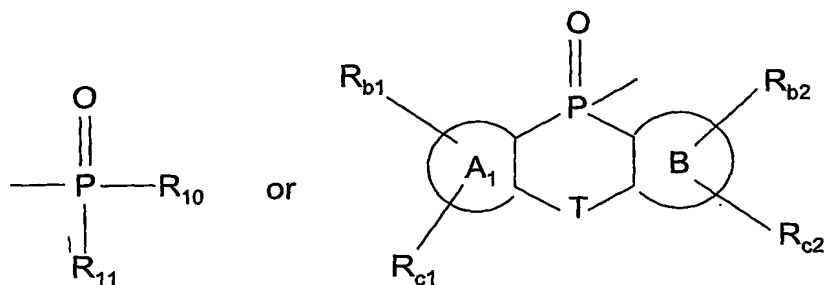
Y_1 is N or CR_{15};

R_{15} is H or lower alkyl;

Q_1 is N or CR_{16};

R_{16} is H or lower alkyl; and

R_{14} is hydrogen, a positively charged electron withdrawing group, SO_2R_{17}, lower alkyl carbonyl, aryl carbonyl, lower arylalkyl carbonyl, BLK_1 AA_1,

R_{10} is OR_{12}, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, cycloalkenyl or cycloalkenyl lower alkyl;

R_{11} is OR_{13}, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, cycloalkenyl or cycloalkenyl lower alkyl; or

R_{10} and R_{11} may optionally be connected by a bridging group T_1 consisting of O, NR_{30} or $(CHR_{30})_m$, wherein R_{30} is lower alkyl or H and m is 1-3;

R_{12} and R_{13} are independently lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl or cycloalkenyl lower alkyl;

rings A_1 and B are independently aromatic rings containing 6 to 14 ring carbon atoms or cycloalkenyl or cycloalkyl, each containing 5 to 14 ring carbon atoms;

T is O, S, NR_{31} or CHR_{31};

R_{31} is H or lower alkyl; and

Rb_1, Rc_1, Rb_2 and Rc_2 are independently hydrogen, lower alkyl or electron donating group;

R_{17} is aryl, loweralkyl or lower arylalkyl, AA_1 is an amino acid or peptide less a hydrogen atom on the N-terminus and an OH group on the C-

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terminus, and BLK$_1$ is an amino protecting group. In addition, the present invention is directed to the use of the compounds of Formula II or, when R$_{14}$ is a positively charged electron withdrawing group, its salt in which the cationic position has the structure of Formula II, in the preparation of peptide.

[0022]   An additional embodiment of the present invention is directed to a process for preparing a peptide bond from the reaction between an amino compound and an acylating derivative of a carboxylic acid, said amino compound being an amino acid or peptide and said carboxylic acid being an N-terminal amino protected amino acid or an N-terminal amino protected peptide, which comprises reacting said amino compound and said acylating derivative of a carboxylic acid in the presence of an effective amount of a compound of formula I or formula II or to a salt, the cationic portion of which has the structure of Formula II, under conditions effective to form a peptide bond.

[0023]   In another embodiment, the present invention is directed to a process for forming an amide from the reaction of an organic amine having a free amino and an acylating derivative of a carboxylic acid, which comprises reacting said amine with the acylating derivative of the carboxylic acid with an effective amount of a compound of Formula I or II or, when R$_{14}$ is a positively charged electron withdrawing group, a salt, in which the cation has the structure of Formula II under amide forming conditions.

[0024]   An additional embodiment of the present invention is directed to the process for synthesizing peptides comprising (a) reacting a first Nα-amino protected amino acid with a peptide synthesis resin under conditions effective to covalently link the amino acid to the resin, (b) cleaving the protecting group from the amino acid to form an amine with a free amino group, (c) reacting said amine with a second N α-amino protected amino acid in the presence of a peptide forming effective amount of a compound of Formula I or II or when R$_{14}$ us a

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positively charged electron withdrawing group, to a salt in which the cation has the structure of Formula II, said reaction being effected under peptide-forming conditions, (d) repeating steps (b) and (c) until the desired peptide is obtained and (e) removing the peptide from the resin.

DETAILED DESCRIPITON OF THE PRESENT INVENTION

[0025]   As used herein, the term "salt of Formula II" or any reference to a salt of Formula II refers to a salt of Formula II which consists of an anion and cation, the cation portion of which is positively charged. The positive charge may result from protonation, such as in the presence of an acid of the compound or Formula II or it may result from R_{14} being a positively charged electron withdrawing group. If reference is to be made to a salt of Formula II in which the structure of Formula II contains a positively charged electron withdrawing group, the term "cation of Formula II" or its equivalent will be used.

[0026]   As described hereinabove, an embodiment of the present invention relates to compounds of Formula I or Formula II or to salts thereof or N-oxide thereof or cation of Formula I and their use in peptide coupling. In other words, a first amino acid or a first peptide, each having a free amino group is coupled with an acylating derivative of either a second amino acid or a second peptide in the presence of compounds of Formula I or II or salts thereof or N-oxides thereof or cation of Formula II under amide forming conditions to form a peptide bond and thus form a larger peptide.

[0027]   As employed herein, the term "heteroaryl" is a heteroaromatic containing at least one heteroatom ring atom selected from nitrogen, sulfur and oxygen and up to a maximum of four ring heteroatoms. The heteroaryl contains from 5 to 14 ring atoms and up to a total of 13 ring carbon atoms and a total of 18 carbon atoms. The heteroaryl group may be monocyclic, bicyclic or tricyclic, although it is preferred that the heteroaryl is bicyclic and especially monocyclic.

Also included in this expression are the benzoheterocyclics. The heteroaryl group preferably contains no more than two ring heteroatoms, and most preferably contains one ring heteroatom. The most preferred ring heteroatoms are oxygen and nitrogen, with nitrogen being the most preferred.

[0028]   If nitrogen is a ring atom, N-oxides can also be formed. The present invention contemplates the N-oxides of the nitrogen containing heteroaryls.

[0029]   Examples of heteroaryls include thienyl, benzothienyl, 1-napthothienyl, thianthrenyl, furyl, benzofuryl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, isoindolyl, indazolyl, purinyl, isoquinolyl, quinolyl, napthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, carbolinyl, isothiazolyl, isothiazolyl and the like. It is preferred that the heteroaryl group is pyridyl, pyrrolyl, furyl, indolyl, quninolyl, isoquinolyl or benzofuryl. Especially preferred is pyridyl.

[0030]   When R_1 and R_2 taken together with the carbons to which they are attached form a tricyclic heteroaryl group, then the compounds of Formula I or II is tetracyclic; if a bicyclic heteroaryl group is formed from R_1 and R_2 taken together with the carbons to which they are attached, then the compounds of Formula I or II are tricyclic. Finally, if R_1 and R_2 taken together form a monocyclic heteroaryl group, then the compounds of Formula I or II are bicyclic. It is preferred that compounds of Formula I and II are tricyclic, and especially bicyclic.

[0031]   The term "heterocyclic", as used herein, when used alone or in combination with other groups, refers to a heterocyclic ring containing at least one heteroatom ring atom selected form nitrogen, sulfur and oxygen up to a maximum of 4 ring heteroatoms and from 5 to 14 ring atoms and up to a total of

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18 carbon atoms. The heterocyclic group may be monocyclic, bicyclic or tricyclic. It may be completely saturated or it may be partially unsaturated, i.e., it may contain one or more double bonds between ring atoms. It is preferred that the heterocyclic group contains 0, 1, 2, 3 or 4 double bonds. The term heterocyclic also includes heteroaryl, as defined herein. Moreover, it is preferred that the heterocyclic moiety contains no more than two ring heteroatoms and most preferably no more than one ring heteroatom. Examples include tetrahydrofuran, morpholinyl, piperazinyl, 2-tetrahydro quinolyl, 3-tetrahydroquinolyl, 6-tetrahydroquinolyl or 7-tetrahydroquinolyl and the like.

[0032]   The term "lower alkyl", when used alone or in combination with other groups, refers to a carbon chain containing from one to six carbon atoms. It may be a straight chain or branched and includes such groups as methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, isobutyl, t-butyl, n-pentyl, amyl, hexyl and the like. The preferred lower alkyl group contains from 1-3 carbon atoms, and is most preferably methyl.

[0033]   The term "aryl" as used herein, alone or in combination, refers to an aromatic ring containing from 6-10 ring carbon atoms and up to a total of 15 carbon atoms. It includes such groups as phenyl, α-naphthyl, β-naphthyl and the like. The preferred aryl is phenyl. It excludes heteroaryls.

[0034]   Aralkyl groups are aryl groups attached to the main chain through an alkylene bridge. Such groups include benzyl, phenethyl and the like.

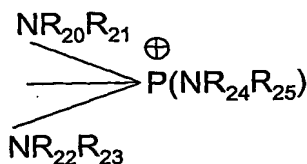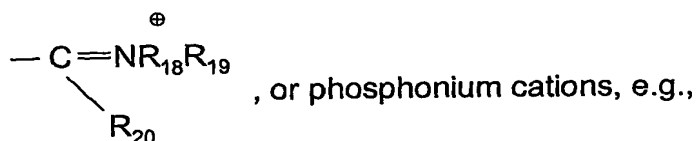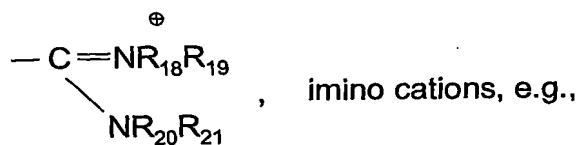[0035]   "Lower alkyl carbonyl" refers to a lower alkyl group attached to the main chain through a carbonyl. Similarly, "aryl carbonyl" refers to an aryl group attached to the main chain through a carbonyl group.

[0036]   "Lower cycloalkyl", as used herein refers to a cycloalkyl group containing 3-10 carbon ring atoms and up to a total of 15 carbon atoms. The cycloalkyl group may be monocyclic or bicyclic or tricyclic. Examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, norbornyl, adamanyl, decalinyl, and the like. The preferred lower cycloalkyl groups are cyclopentyl and cyclohexyl.

[0037]   "Lower cycloalkyl lower alkyl" is a lower cycloalkyl group attached to the main chain through an alkylene bridge. Such groups include cyclohexylmethyl, cyclopentylethyl and the like.

[0038]   "Cycloalkenyl" refers to a lower cycloalkyl group, as defined herein, containing at least one double bond and up to a maximum of 6 carbon-carbon double bonds. It is not completely aromatic; but it may include an aromatic moiety. It may contain one ring or two or more rings fused together. The double bonds may be located in one ring or both rings. One or more rings may be completely aromatic, while the remaining rings, if any, in the structure may each be completely saturated or contain 1 or 2 double bonds. It is to be noted however, that cycloalkenyl, as described herein, excludes aryl. Examples include cyclohexenyl, cyclooctenyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8- indanyl, and the like.

[0039]   As used herein, an "electron donating group" shall designate a group that will release or donate electrons more than hydrogen would if it occupied the same position in the molecule. See., J. March, Advanced Organic Chemistry, 3rd Ed., John Wiley & Sons p. 237 (1985). These types of groups are well known in the art. Examples include lower alkylamino, diloweralkylamino, amino, halo, aryl, lower alkoxy, lower aralkoxy, aryloxy, mercapto, lower alkylthio, and the like. The preferred electron donating groups are amino, hydroxy, lower alkoxy, lower alkylamino and diloweralkylamino.

[0040]   The term "electron withdrawing groups" as defined herein refers to a group that will draw electrons to itself more than a hydrogen atom would if it occupied the same position in the molecule. See., J. March, Advanced Organic Chemistry, 3rd Ed., John Wiley & Sons p. 237 (1985). They include such groups as nitro, monohaloalkyl, dihaloalkyl, trihaloalkyl (e.g., CF_3), halo, formyl, lower alkanoyl, lower alkylsulfonyl, lower alkylsulfinyl, carboxy, lower alkoxy carbonyl, sulfonamido, amido, $CONR_{50}R_{51}$, wherein R_{50}, and R_{51} are independently hydrogen lower alkyl, aryl, aryl lower alkyl, heterocyclic; heterocyclic lower alkyl, lower cycloalkyl, lower cycloalkyl lower cycloalkyl lower alkyl, cycloalkenyl cycloalkenyl lower alkyl and the like.

[0041]   A "positively charged electron withdrawing group" is an electron withdrawing group bearing a positive charge and forming a stable bond to a N-hydroxide (N-O). These types of groups are well known in the art. Examples include uronium groups,
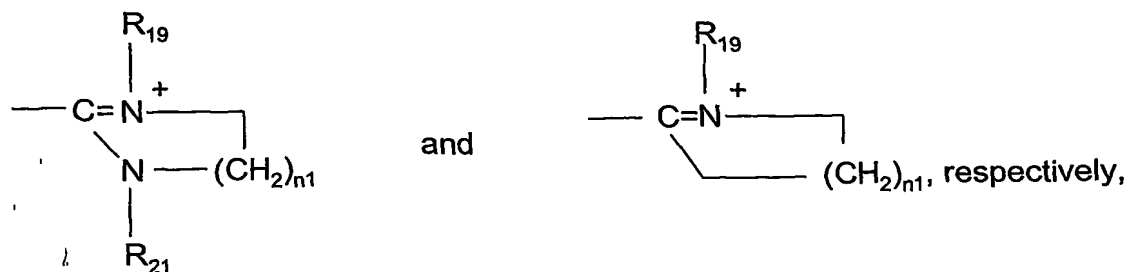
e.g., $-C\overset{\oplus}{=}NR_{18}R_{19}$
            $\diagdown$
                 $NR_{20}R_{21}$, imino cations, e.g.,

$-C\overset{\oplus}{=}NR_{18}R_{19}$
    $\diagdown$
         R_{20} , or phosphonium cations, e.g.,

$NR_{20}R_{21}$
$\diagdown$
      $\overset{\oplus}{\diagup}P(NR_{24}R_{25})$
$\diagup$
$NR_{22}R_{23}$ and the like, wherein R_{18}, R_{19}, R_{20}, R_{21}, R_{22}, R_{23}, R_{24} and R_{25} are independently hydrogen , lower alkyl, lower alkoxy lower alkyl or if the imino cation is formed, R_{18} ad R_{20} taken together with the nitrogen atoms to which they are attached and the carbon atom therebetween may form a ring containing up to 6 ring atoms and up to a total of 5 ring carbon atoms or R_{18} and R_{19} taken together with the nitrogen atom to which they are attached or R_{20} and R_{21} taken

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together with the carbon atoms to which they are attached may form a 5 or 6-membered heterocyclic ring containing up to a total of 5 ring carbon atoms or if the uronium cation is formed, R_{20} and R_{18} may be taken with the nitrogen to which they are attached form a 5 or 6 membered heterocyclic ring containing up to a total of 5 ring carbon atoms or both R_{18} and R_{19} taken together with the nitrogen atoms to which they are attached or R_{20} and R_{21} taken together with the nitrogen atom to which they are attached, may each simultaneously form a 5 or 6-memebered heterocyclic ring , each ring containing up to a total of 5 ring carbon atoms. In the uronium and imino cations, it is preferred that R_{18} and R_{19} and R_{20} and R_{21}, when present, are the same. It is especially preferred that R_{18}, R_{19}, R_{20}, R_{21}, whenever present, are the same. It is also more preferred that the rings formed with respect to the uronium, imino, and phosphonium cations are 5 or 6 membered rings.

[0042]   With respect to the phosphonium cation, R_{18} and R_{19} and/or R_{20} and R_{21} and/or R_{22} and R_{23} may each be independently taken together with the nitrogen atoms to which they are attached to form a ring. Thus, the phosphonium cation may be comprised of 1 ring, two rings or three rings. It is preferred that R_{18} and R_{19}, or R_{20} and R_{21} or R_{22} and R_{23} are the same. It is especially preferred that R_{18}, R_{19}, R_{20}, R_{21}, R_{22} and R_{23} are the same.

[0043]   With respect to cations of Formula II, preferred cyclic uronium and imino groups have the formula

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$$\begin{array}{c} R_{19} \\ | \\ -C=N^+- \\ \diagdown \\ N-(CH_2)_{n1} \\ | \\ R_{21} \end{array} \quad \text{and} \quad \begin{array}{c} R_{19} \\ | \\ -C=N^+- \\ \diagdown \\ (CH_2)_{n1} \end{array}, \text{ respectively,}$$
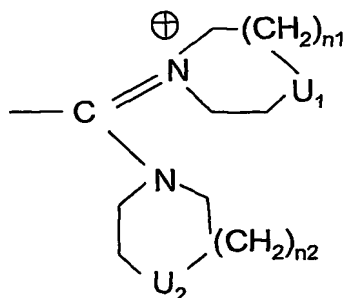
wherein R_{19} and R_{20} are as defined hereinabove and n_1 and n_2 are independently 0 or 1, and U_1 and U_2 are independently O, CH_2 or NH or N-Alk wherein Alk is lower alkyl.

[0044]   In the above formulae, the preferred values of R_{18}, R_{19}, R_{20} R_{21}, R_{22} and R_{23} are independently methyl, ethyl, n-butyl, pentyl and $-CH_2CH_2-O-CH_2CH_3$. It is preferred that R_{18}, R_{19}, R_{20} (for all) and R_{21}, when present (for imino), and R_{22} and R_{23} (for phosphonium) when present, are the same.

[0045]   The preferred values of R_{18}, R_{19}, R_{20}, R_{21}, when present, and R_{22}, and when present, and R_{23}, when present, are independently lower alkyl, especially methyl. It is preferred R_{18}, R_{19}, R_{20} and R_{21}, when present, R_{22}, when present, and R_{23}, when present, are all the same. Further it is preferred that all are methyl.

[0046]   With respect to the uronium and/or imino cations, when R_{18} and R_{19} or R_{20} and R_{21} taken together form a ring, they may form heterocyclic moieties of the formula:

$$-N\diagup\diagdown U \quad \text{or} \quad \diagup\diagdown N- , \text{ wherein}$$
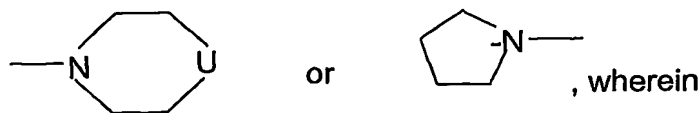
$U=CH_2$, O, NH or N-Alk, wherein Alk is lower alkyl, especially methyl.

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[0047]   Preferred cyclic groups present in the phosphonium cations also have the formula

 or , wherein U, is defined hereinabove.
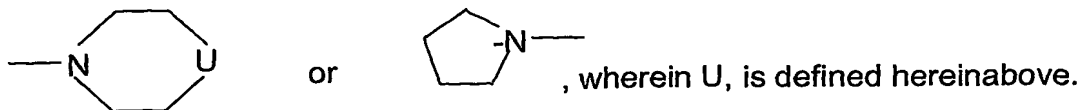
[0048]   It is preferred that R_1 and R_2 taken together with the carbon atoms to which they are attached form an aryl or heteroaryl ring wherein the aryl ring is a phenyl or naphthyl and the heteroaryl ring contains 5 to 10 ring atoms and one or two ring heteroatoms consisting of O, S, N and 3-8 ring carbon atoms.

[0049]   With respect to compounds of Formula I, the following are preferred. When n is 1, it is preferred that R_7 and R_8 are hydrogen or lower alkyl, but most preferably hydrogen.

[0050]   Preferred values of Y are S, O, NR_4, or CR_4R_5, wherein R_4 and R_5 are independently hydrogen or methyl. Especially preferred values of Y are O, CH_2 and NH. It is also preferred, however, that Q is absent and Y forms a double bond with X.

[0051]   It is preferred that X is CR_6R_7 or NR_6. Preferred values of R_6 and R_7 are hydrogen or lower alkyl.

[0052]   With respect to compounds of Formula I, it is preferred that Q is not present and Y and X are independently N or CH. It is more preferred that at least one of Y and X is N and the other is CH. It is even more preferred that Y is N and X is CH or N or that Y and X are both N.

[0053]   When R_6 and R_7 taken together form an oxo group, X becomes C = O. It is most preferred that X is C = O, CH_2 or NH or $N(CH_3)$. Moreover, an embodiment of Formula I has the formula

19
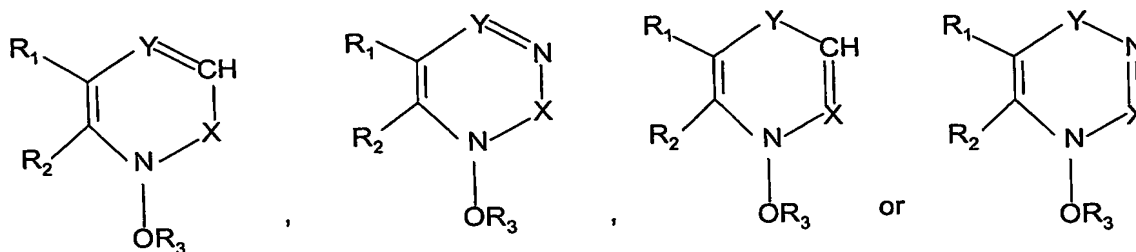
[0054]   In cases when n is 0, then R_4 and R_6 taken together may form a bond between X and Y, i.e., a bond may form between the ring carbon atom of X and the ring carbon atom of Y, or between the ring nitrogen atom of X and the ring nitrogen atom of Y, or the ring nitrogen atom of X, and the ring carbon atom of Y or the ring carbon atom of X and the ring nitrogen atom of Y. In other words, under these circumstances when n is O and R_4 and R_6 taken together form a bond between X and Y, the compound of Formula I becomes
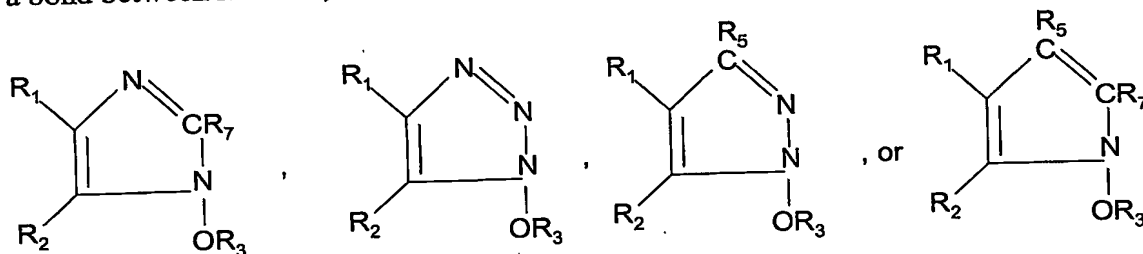
wherein R_1, R_2, Y, X, and R_3 are as defined above. Under these circumstances, it is preferred that Y is CH or N and X is CH or N. It is most preferred that Y and X are N.

[0055]   When n is 1, the compound of Formula I becomes
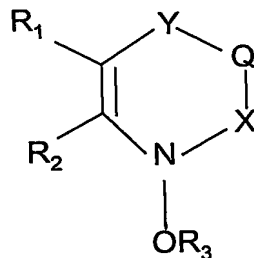
wherein

R_1, R_2, Q, R_8, R_9, Y, R_4, R_5, X, R_6, R_7 and R_3, R_{10}, R_{11}, R_{12} and R_{13}

are as defined above. It is preferred that R_8 and R_9 are hydrogen.

[0056]   As indicated hereinabove, when n is 1, R_4 and R_8 taken together may form a bond between Q and Y, i.e., the ring carbon atom of R_4 and the ring carbon atom of R_8 may form a bond, or the ring carbon atom of R_4 and the ring nitrogen atom of R_8 may form a bond, or the ring nitrogen atom of R_4 and the ring carbon atom of R_8 may form a bond or the ring nitrogen atom of R_4 and the ring nitrogen atom of R_8 may form a bond. For example, under these circumstances, the compound of Formula I becomes:
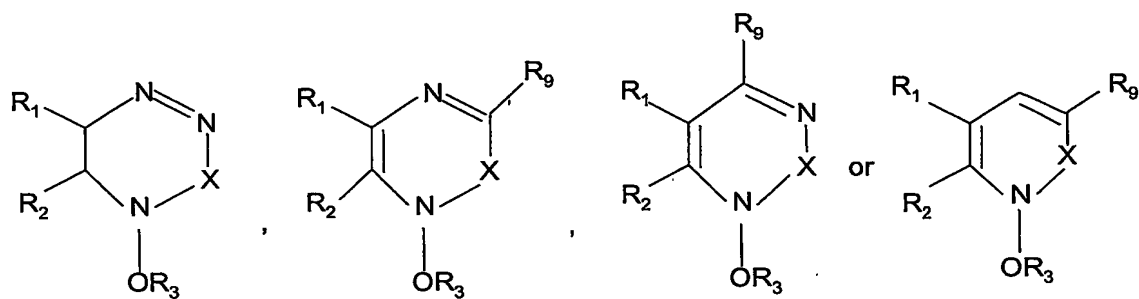
[0057]   The preferred values of X in this formulations are C =O or NH or CH$_2$.

[0058]   When n is 1, preferred values of Q are CH$_2$ or NH. However, it is also preferred that the compounds of Formula I have the formula:

wherein

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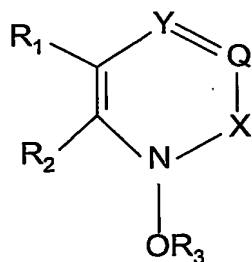
Q is CR$_9$ or N, and

R$_9$ is hydrogen or lower alkyl

and R$_1$, R$_2$, X, OR$_3$ and Y are as defined hereinabove. Examples of compounds of
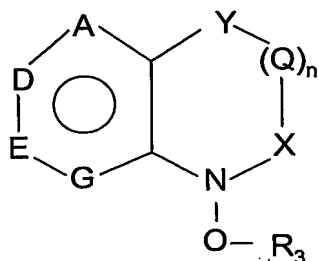
Formula I include:
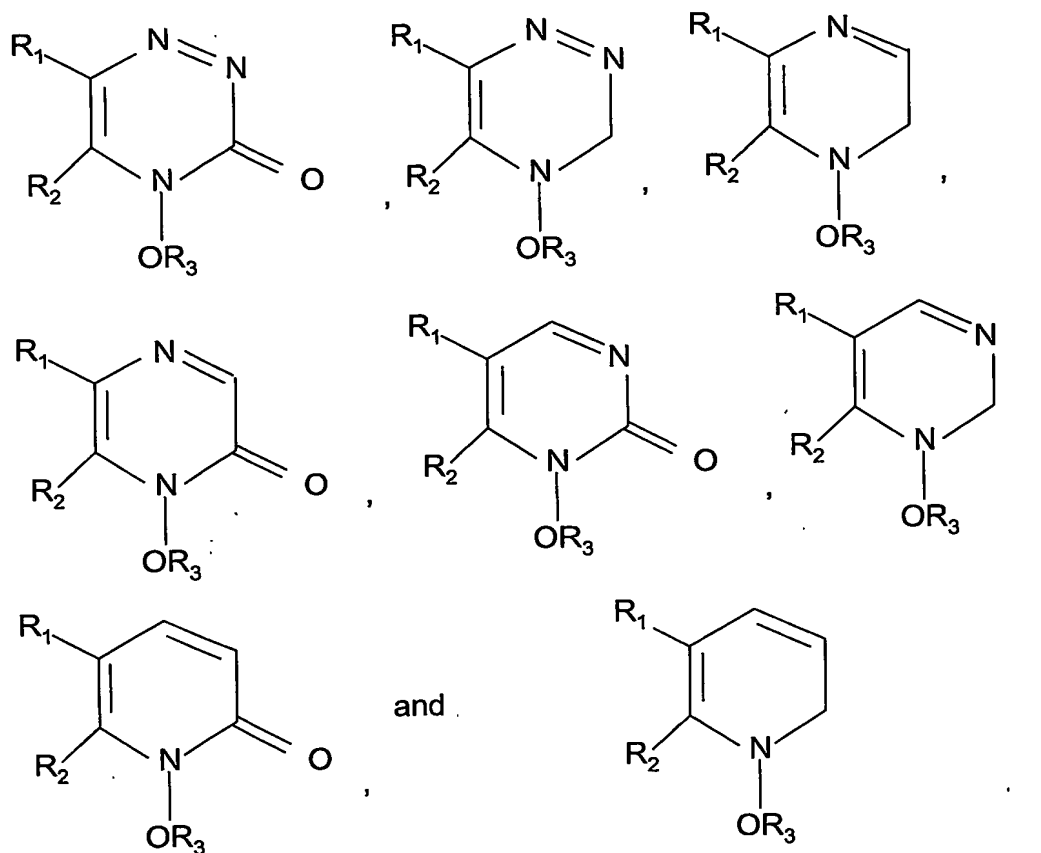
wherein R$_1$, R$_2$ and R$_3$ are as defined hereinabove.

[0059]   It is also preferred that compounds of Formula I have the formula:
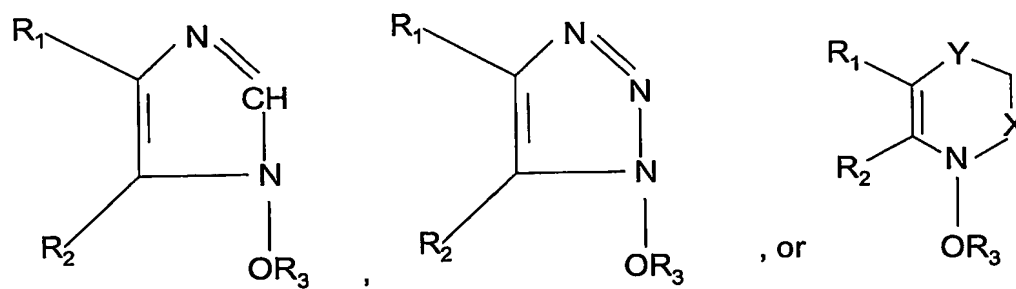
wherein R_1, R_2, R_3, Y and X are as defined hereinabove.

[0060]   The most preferred compounds of Formula I have the formula:

III

or N-oxides thereof

wherein Q, Y, X, R_3, n, are as defined hereinabove,

A is N or CR_{24};

D is CR_{25} or N;

E is CR_{26} or N;

G is CR_{27} or N; and

R_{24}, R_{25}, R_{26} and R_{27} are independently hydrogen or lower alkyl or an electron donating group or R_{25} and R_{26} or R_{24} and R_{25} or R_{26} and R_{27} taken together form with the carbon atom to which they are respectively attached an aryl ring. It is preferred that A, D, E or G all are CH and more preferably at least one of A, D, E, G is N.

[0061]   It is preferred that no more than two of A, D, E, G are N. It is most preferred that only one of A, D, E, G is N. Further it is preferred that R_{24}, R_{25}, R_{26} or R_{27} are hydrogen or an electron-donating group, as defined herein, The preferred electron donating group is lower dialkylamino especially N, N-dimethyl amino and lower alkoxy, e.g. methoxy.
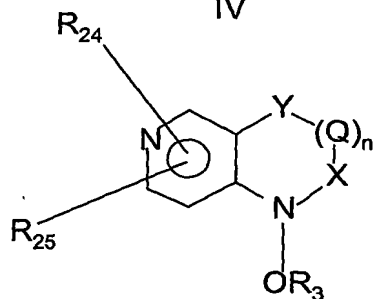
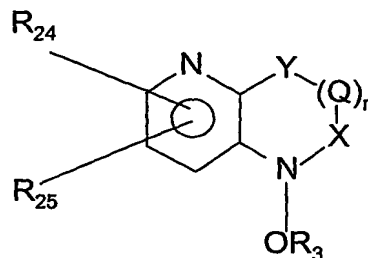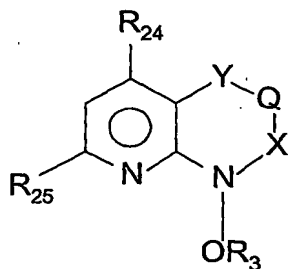[0062]   Preferred compounds of Formula III have the formulae:

R_{24}

R_{25}

OR_3

IV

R_{24}

R_{25}

OR_3

V

R_{24}

R_{25}

OR_3

VI

R_{24}

R_{25}

OR_3
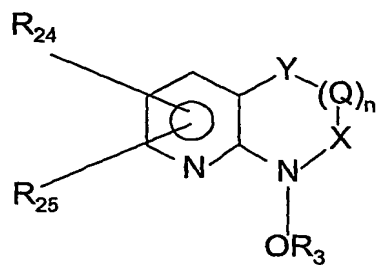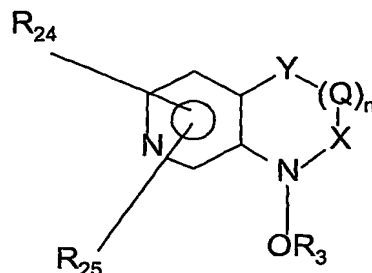
VII

or N-oxides thereof

wherein Y, X, n, Q and R_3 are as defined hereinabove and R_{24} and R_{25} are independently lower alkyl, hydrogen or an electron donating group.

[0063]   Of the compounds of Formula IV-VII, when n is 1, the most preferred compound is that of Formula IVa
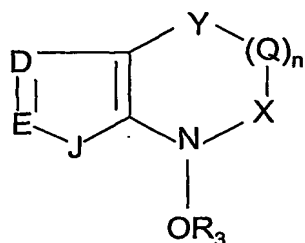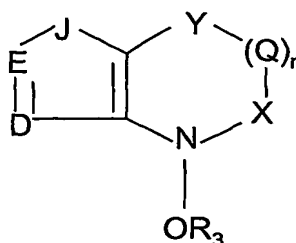
R_{24}

R_{25}

OR_3

IV A

or N-oxides thereof

wherein Q, Y, X and R_3 are as defined hereinabove and R_{24} and R_{25} are lower alkyl or hydrogen or an electron donating group.

[0064]   Preferred compounds of Formula I also have the formula
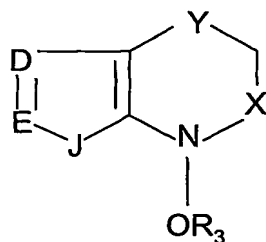
VIII   IX

or N-oxides thereof

wherein n, Q, D, E, X and Y are as defined hereinabove and J is NR_{28}, O, $CR_{28}R_{29}$ or S(O)p, and p is 0, 1, 2.

R_{28} is hydrogen , lower alkyl or electron donating group as defined hereinabove and R_{29} is hydrogen or lower alkyl. It is preferred that R_{29} is hydrogen. The preferred values of R_{28} is an electron donating group or hydrogen.

[0065]   Preferred values of J are O or S(O)p; the preferred value of p is 1.

[0066]   Preferred compounds of Formula VIII when n is 1 have the formula:

                         VIIIa

or N-oxides thereof

wherein J, Y, R_8, R_9, n and R_3 are as defined hereinabove and X is C =O.

[0067]   In compounds VIII, IX, and VIIIa as depicted above, at least one of D, E, or J is a heteroatom. Furthermore, it is most preferred that at most two of J, E, and D are heteroatoms. It is most preferred that only one of J, E, and D is a heteroatom.

25

[0068]   When n is O, preferred compounds of Formula I becomes:
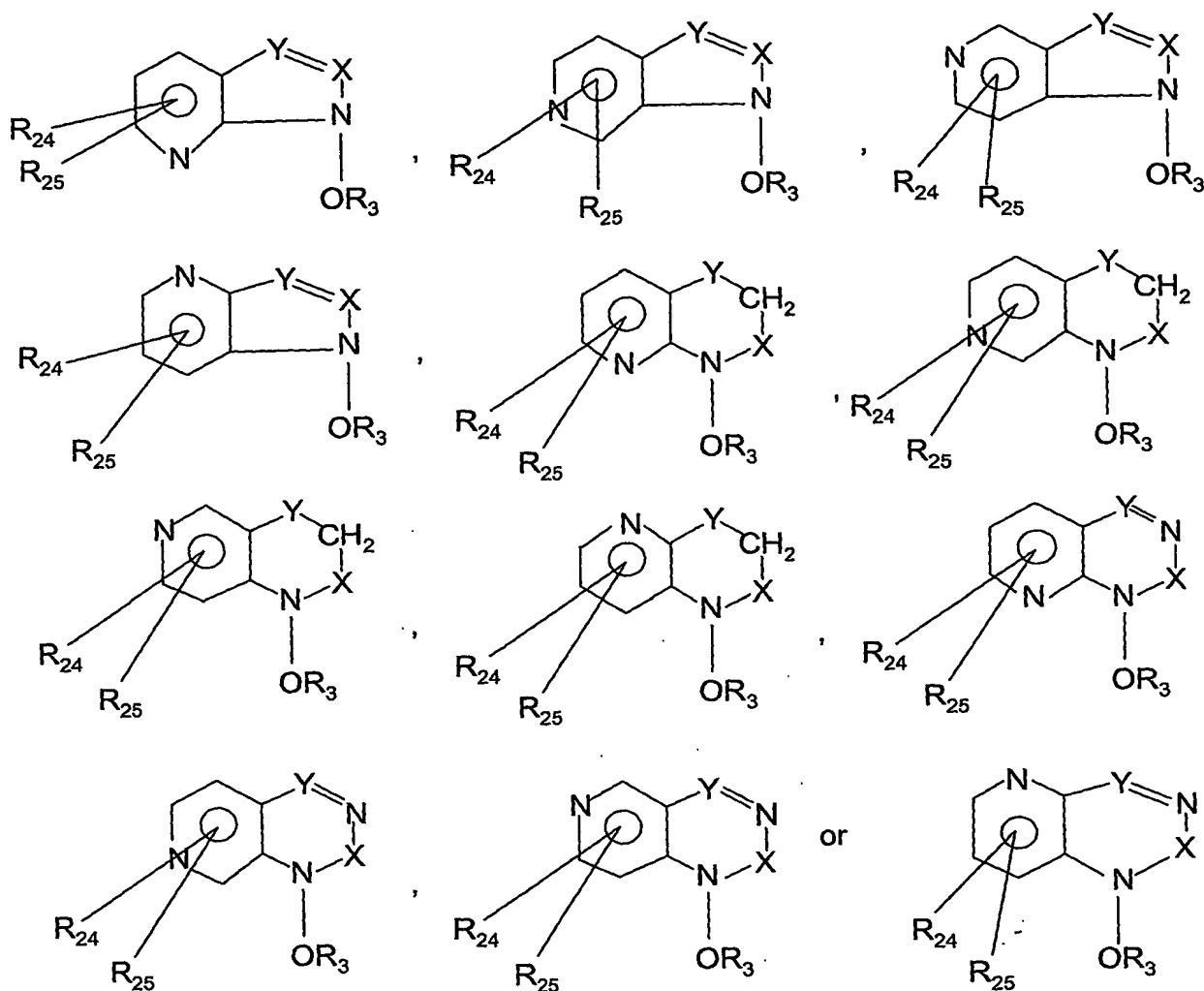

or
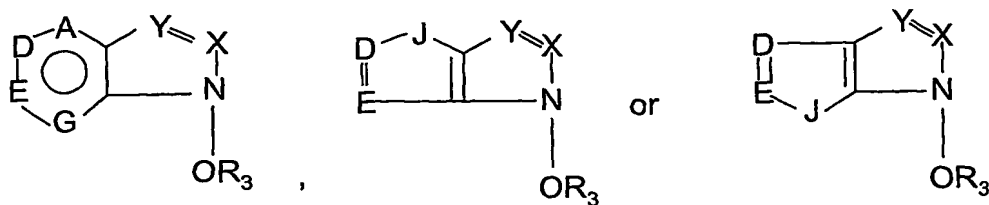
[0069]   Thus, the present invention includes compounds having the

formula: ,
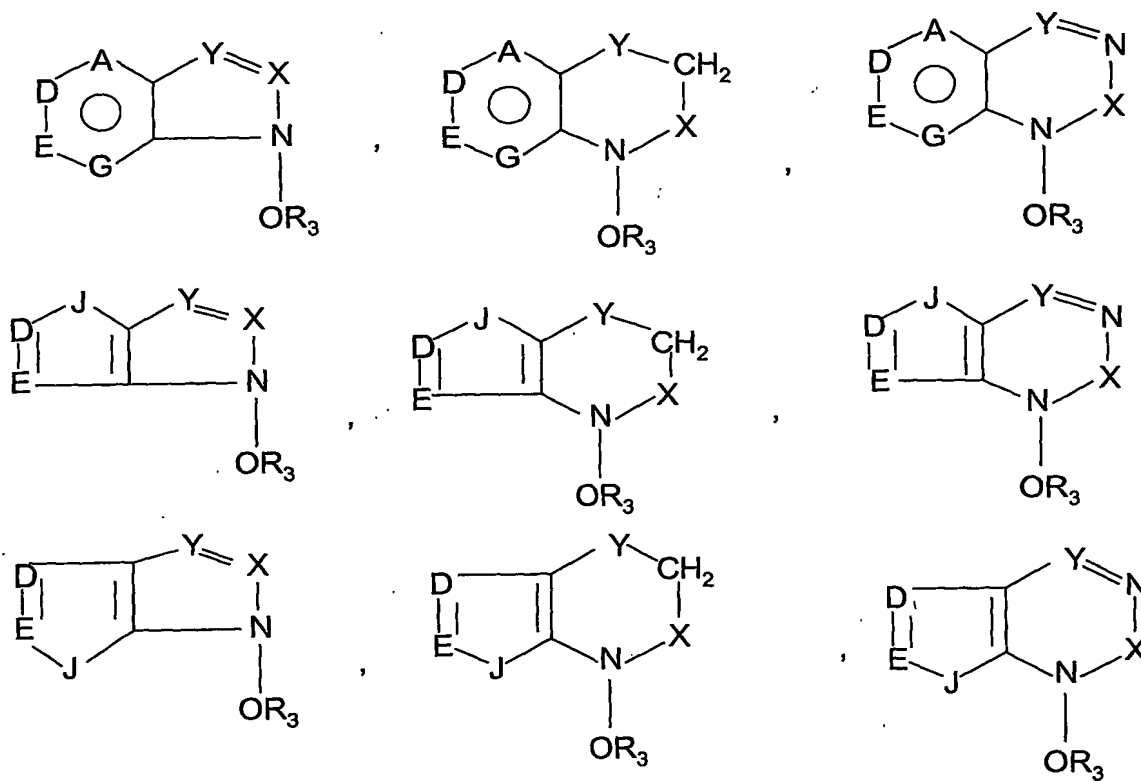
or N-oxides thereof.

wherein A, D, E, G, Y, X, R₃ and J are as defined hereinabove.

[0070]   The compounds of Formula I more preferably are compounds of

the formula:

26

or N-oxides thereof.

[0071]   In the above formulae, when the ring contains Y =X, this means that R_4 of Y and R_6 of X are joined together to form a ring bond betweent the Y ring atom and the X ring atom, so that as depicted hereinabove there is a double bond between the Y ring atom and the X ring atom.

[0072]   Furthermore, in the above formulae, when the ring contains Y = N, then R_4 of Y and R_8 of NR_8 of Q join together to form a ring bond so that there is

27

a double bond between the nitrogen ring atom and the Y atom. Thus, Y is CR$_5$ or N under these circumstances.

[0073]   The most preferred embodiment of Formula I has the formula:
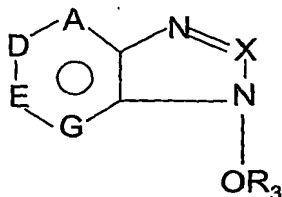
wherein one or two of A, D, E, G, is N and the rest are CH and X is CH or N. It is most preferred that X is N. It is also preferred that at most one of A, D, E and G is N and the rest are CH. It is most preferred that A is N and especially G is N.

[0074]   Preferred embodiments of compounds of Formula I include:
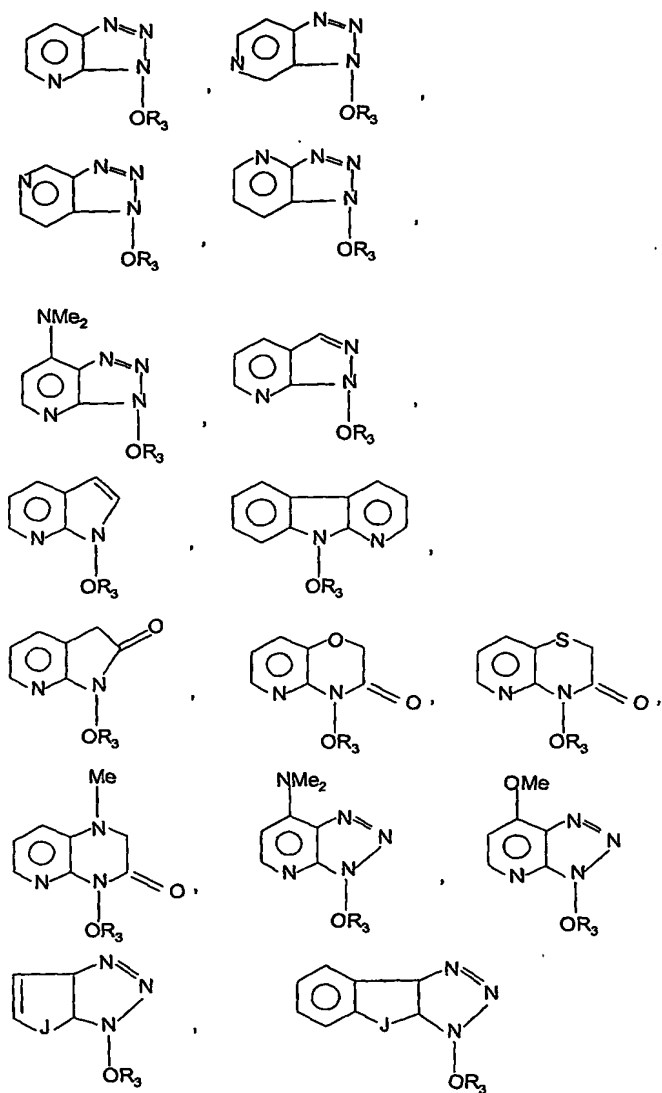
or the N-oxides thereof.

[0075]   With respect to compounds of Formula I, R₃ is as defined
hereinabove. The various groups on R₁₀, R₁₁, R₁₂ and R₁₃, e.g., alkyl, aryl,
arylalkyl, cycloalkyl, cycloalkylalkyl, heterocyclic, heterocyclic lower alkyl or
lower cycloalkyl heterocyclics may be unsubstituted, or substituted by lower alkyl
or electron donating or electron withdrawing groups. It is preferred that the
groups are unsubstituted or substituted by lower alkyl.

[0076]   With respect to compounds of Formula I, it is preferred that R_{10} is OR_{12}, lower alkyl, aryl or aryl lower alkyl. It is more preferred that R_{10} is OR_{12} or aryl.

[0077]   It is also preferred that R_{11} is OR_{13}, lower alkyl, aryl or aryl lower alkyl. It is most preferred that R_{11} is OR_{13} or aryl. Preferred values of R_{12} and R_{13} each independently are lower alkyl, aryl or aryl or aryl lower alkyl. It is most preferred that R_{12} and R_{13} are alkyl having 1-3 carbon atoms, or phenyl. It is also preferred that R_{12} and R_{13} are the same.

[0078]   It is also preferred that R_{10} and R_{11} are connected to each other by a bridging group, T_1. Preferred values of T_1 are O, CH_2, S, or NR_{30} when R_{30} is lower alkyl and more preferably H. When R_{10} and R_{11} are joined together, then R_3 becomes
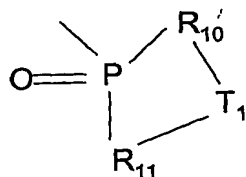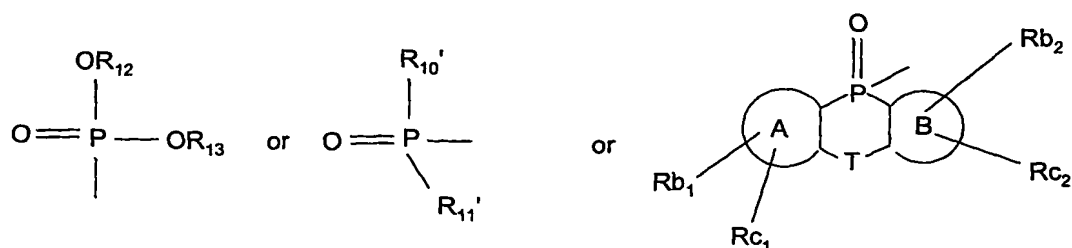

wherein R_{10}, R_{11} and T, are as defined hereinabove. As defined herein R_3 may be defined as
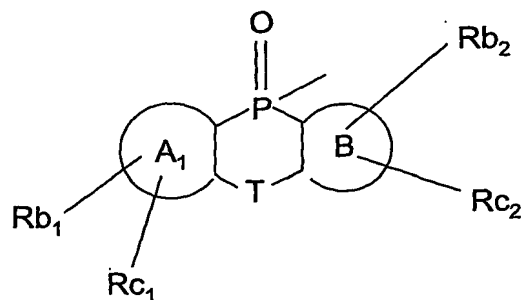
[0079]   It is preferred that rings A_1 and B are independently aromatic, especially phenyl.

[0080]   It is preferred that R_3 is

wherein R_{10}' and R_{11}', R_{12} and R_{13} are independently lower alkyl, aryl or aryl lower alkyl and Rb_1, Rc_1, Rb_2 and Rc_2 are independently hydrogen or lower alkyl. It is also preferred that R_{12} and R_{13} are connected by a bridging group T_1 to form the following R_3 moiety
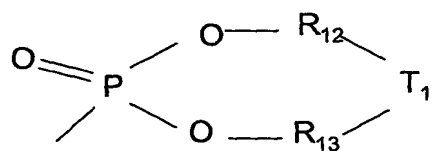
[0081]   In an embodiment of the present invention, R_{10} and R_{11} are connected by the bridging group T_1, as depicted hereinabove. Preferred values of T_1 are CH_2, O, S and NH and most preferably CH_2 and O.

[0082]   Embodiments of R_3 include

, and the
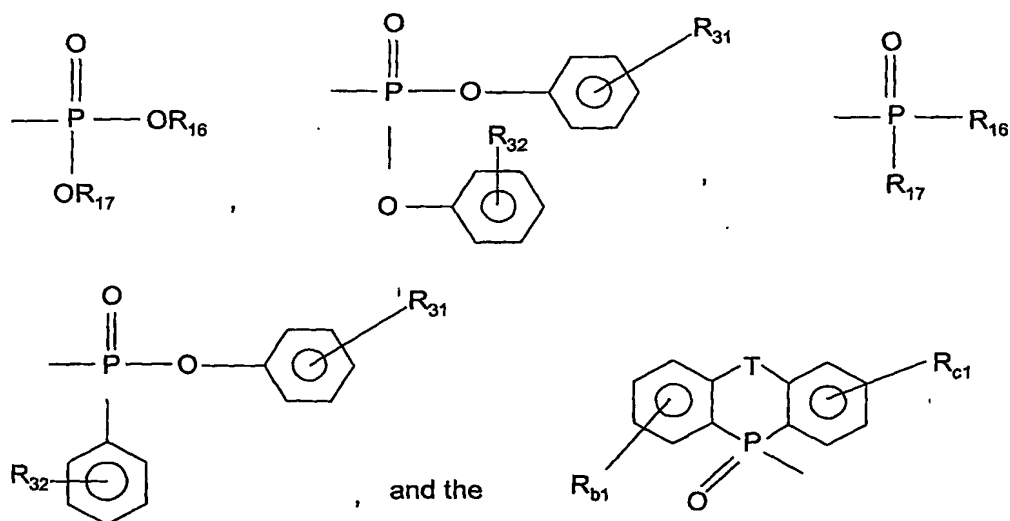
wherein R_{16} and R_{17} are independently lower alkyl, R_{b1} and R_{c1} are independently H or lower alkyl and T is as defined hereinabove and preferably O, NH or CH_2.

[0083]   Examples of R_3 include

and
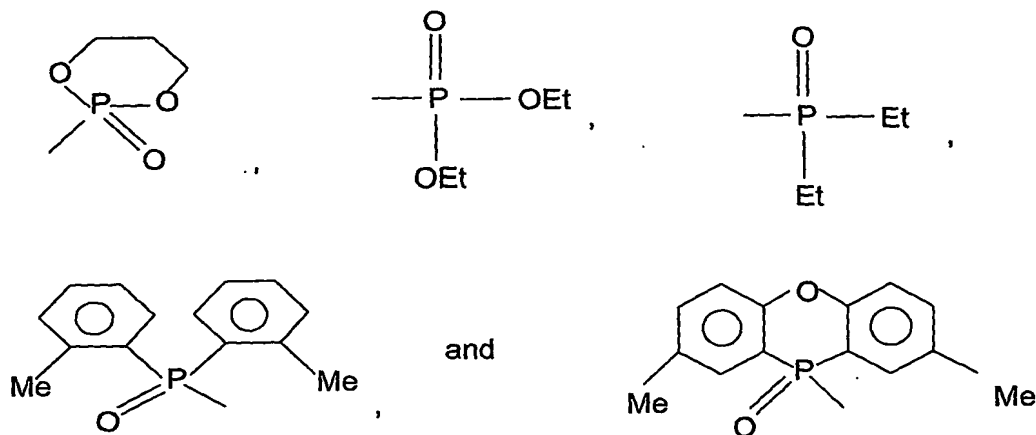
[0084]   Of course, various combinations and permutations of the formulae described herein are also contemplated by the present invention. In addition, Markush groupings containing less than all of the elements described hereinabove as well as the various permutations thereof are also contemplated by the present invention.

[0085]   The compounds of Formula I can be prepared by art recognized techniques. An illustrative technique is described hereinbelow.

[0086]   For example, compounds of formula

can be prepared by reacting

100
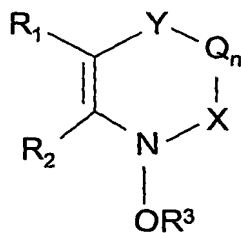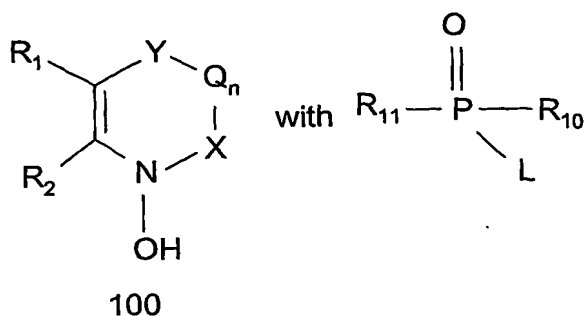
under substitution reaction conditions wherein R_1, R_2, Y, Q, n, R_3, R_{10} and R_{11} are as defined hereinabove and L is a leaving group, such as halo and the like.

[0087]   It is preferable that the reaction is run in an inert polar organic solvent and that the reactants are soluble therein at room temperature. It is also preferred that the product of the substitution is insoluble in the solvent at room temperature. Examples of suitable solvents for the reaction include chloroform, carbon tetrachloride, ethyl ether, dioxane, tetrahydrofuran and methylene dichloride, and the like. The reaction takes place at effective temperatures, which may range from the melting point of the solvent to reflux temperature thereof but it is preferred that the reaction take place at about room temperature or at slightly elevated temperatures up to the reflux temperature of the solvent. It is especially preferred that the reaction take place at room temperature or at slightly elevated temperatures, such as up to 60°C.

[0088]   Compounds of Formula 100 can be prepared as described in U.S. Patent No. RE 37,686, RE 38,073, 5,580,981, 5,644,029, 5,698,675, the contents of which are incorporated by reference.

[0089]   For example, compounds of Formula 100, such as
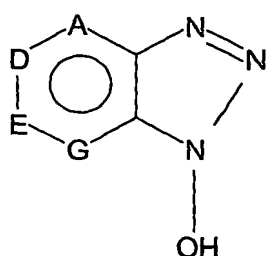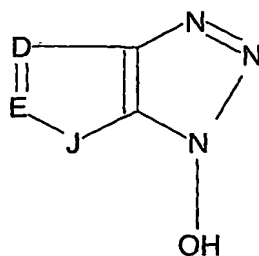
100(a)   100(b)

and
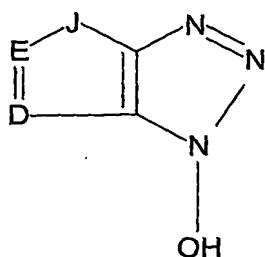
100(c)

can be prepared by reacting hydrazine with

101(a)   101(b)   101(c)
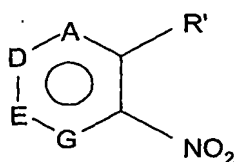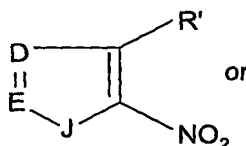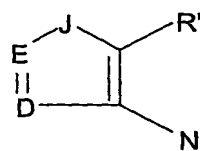
wherein R' is halogen, NH-NH$_2$ or OR", and

R" is lower alkyl, such as methyl. This reaction is performed at slightly elevated temperatures, such as 70-100°C, although the reaction may be performed at temperatures ranging from room temperature to the boiling point of the solvent.

[0090] The reaction is usually run in an organic solvent in which the reactants are insoluble at room temperature, but in which the reactants and product are soluble at slightly elevated temperatures. Examples of useful solvents include ethanol, DMF and the like. In many cases, there is a color change in the

reaction mixture, indicating the formation of the product. Work-up, such as removal of the solvent, followed by acidification provides the desired product.

[0091]   The hydrazino derivative ($R' = NH\text{-}NH_2$) can be prepared by reacting the corresponding halide, such as chloride or bromide, with hydrazine under substitution reaction conditions. The ether derivative ($R^1 = OR''$) can be prepare by reacting the corresponding alcohol with an alkylating reagent, such as Me_2SO_4/Na_2CO_3, under ether forming conditions.

[0092]   Compounds of Formulae 100a, 100b, or 100c are useful for preparing compounds of Formula I. These latter compounds can also be prepared by art-recognized techniques. For example, compounds of Formula I are prepared by reacting compounds of 100a, 100b, 100c, respectively, with

$$R_{11}\underset{\underset{\displaystyle L}{\diagdown}}{\overset{\overset{\displaystyle O}{\|}}{P}}R_{10}$$
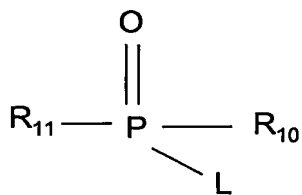
under substitution reaction conditions wherein R_{10} and R_{11} are defined hereinabove and L is a leaving group, such as halo, (e.g., Cl, Br, I) and the like.

[0093]   The N-oxides can be prepared from the compounds of Formula I having a nitrogen ring heteroatom in the heteroaryl group. These N-oxides are prepared by art-recognized techniques such as by oxidation thereof, such as with peracid, e.g., peracetic acid or m-chloroperbenzoic acid.

[0094]   With respect to compounds of Formula II, it is preferred that Y_1 is N or CR_{15}, wherein R_{15} is hydrogen or methyl. Especially preferred values of Y_1 are CH and N.

[0095]   It is also preferred that Q_1 is N or CR_{16} wherein R_{16} is hydrogen or lower alkyl. The preferred value of Q_1 is N or CH.

[0096]   With respect to compounds of Formula II, preferred values of Y_1 and Q_1 are CH or N. In a preferred embodiment, the compound of Formula II has the formula
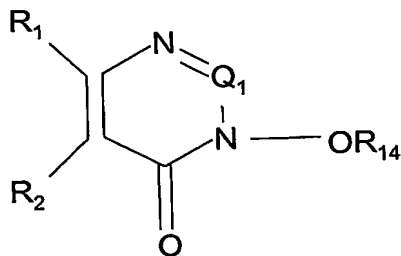
wherein R_1 and R_2 and R_{14} are as defined as hereinabove and Q_1 is N or CH, but especially N.

[0097]   Another embodiment of the compound having Formula II or salt of Formula II, wherein the cation has the formula is:
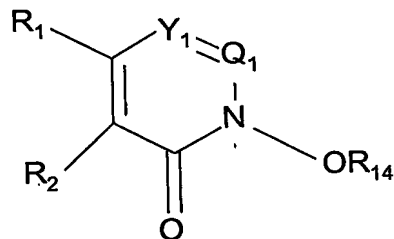
wherein

R_1 and R_2 taken together with the carbon atoms to which they are attached form an heteroaryl ring wherein said heteroaryl ring is an oxygen, sulfur or nitrogen heteroaromatic containing from 3 to 13 ring carbon atoms and 1-4 heteroatoms selected from O, S and N, said heteroaryl ring may be unsubstituted or substituted with lower alkyl or electron donating group;

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Y_1 is N or CR_{15};

R_{15} is H or lower alkyl;

Q_1 is N or CR_{16};

R_{16} is H or lower alkyl;

R_{14} is hydrogen, a positively charged electron withdrawing group,
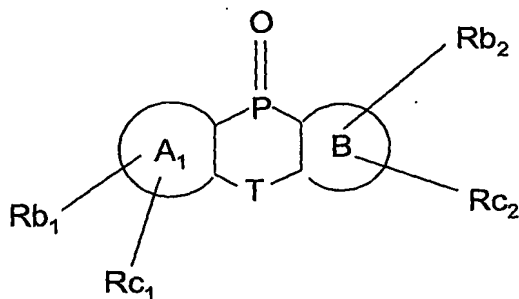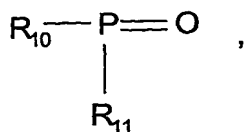
SO_2R_{17}, lower alkyl carbonyl, aryl carbonyl, loweralkyl aryl, or BLK_1-AA_1

R_{17} is aryl, aryl lower alkyl or lower arylalkyl;

AA_1 is an amino acid or peptide less a hydrogen atom on the N-terminus and an OH on the C-terminal;

BLK_1 is an amino protecting group,

R_{10} is OR_{12}, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl, lower cycloalkenyl or lower cycloalkenyl lower alkyl;

R_{11} is OR_{13}, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl, lower cycloalkenyl

lower alkyl;

and R_{10} and R_{11} may optionally be connected by a bridging group selected from the group consisting of O, S, NR_{30}, or $(CHR_{30})_m$, wherein each R_{30} is independently lower alkyl or hydrogen and m is 1-3;

R_{12} and R_{13} are independently lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl, or lower cyclalkenyl lower alkyl;

ring A_1 and ring B are independently aromatic containing 6 to 14 ring carbon atoms or cycloalkenyl or cycloalkyl, each containing 5 to 14 ring carbon atoms;

R_{b1}, R_{c1}, R_{b2}, R_{c2} are independently hydrogen, lower alkyl or electron donating group;

T is CHR_{31}, O, S or NR_{30}; and

R_{31} is hydrogen or lower alkyl.

[0098]   Another embodiment of the compound having Formula II or salt of Formula II, wherein the cation has the formula is:
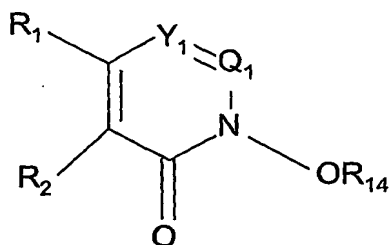
or N-oxide or salt thereof

wherein one of Y_1 and Q_1 is CR_{15} and the other is N or CH;

R_{15} is H or lower alkyl;

R₁ and R₂ taken together with the carbon atom to which they are

attached form an aryl or heteroaryl ring wherein said aryl ring is an aromatic ring

containing 6-14 ring carbon atoms and said heteroaryl ring is an oxygen, sulfur or

nitrogen heteroaromatic containing from 3 to 13 ring carbon atoms and 1-4

heteroatoms selected from O, S and N, said heteroaryl ring may be unsubstituted

or substituted with lower alkyl or electron donating group;

R₁₄ is hydrogen, a positively charged electron withdrawing group,
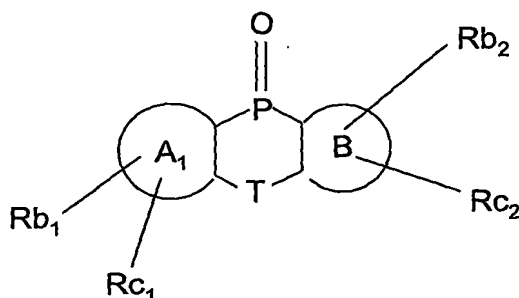
SO₂R₁₇, lower alkyl carbonyl, aryl carbonyl, loweralkyl aryl, or BLK₁-AA₁

R₁₇ is aryl, aryl lower alkyl or lower arylalkyl;

R₁₁ is OR₁₃, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl

heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl, lower cycloalkenyl

lower alkyl;

R₁₀ is OR₁₂, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl,

lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower

cycloalkenyl, lower cycloalkenyl or lower cycloalkenyl lower alkyl;

and R₁₀ and R₁₁ may optionally be connected by a bridging group

selected from the group consisting of O, S, NR₃₀, or (CHR₃₀)ₘ, wherein each R₃₀

is independently lower alkyl or hydrogen and m is 1-3;

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AA$_1$ is an amino acid or peptide less a hydrogen atom on the N-terminus and an OH on the C-terminal;

BLK$_1$ is an amino protecting group,

R$_{12}$ and R$_{13}$ are independently lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl, or lower cyclalkenyl lower alkyl;

ring A$_1$ and ring B are independently aromatic containing 6 to 14 ring carbon atoms or cycloalkenyl or cycloalkyl, each containing 5 to 14 ring carbon atoms;

R$_{b1}$, R$_{c1}$, R$_{b2}$, R$_{c2}$ are independently hydrogen, lower alkyl or electron donating group;

T is CHR$_{31}$, O, S or NR$_{31}$; and

R$_{31}$ is hydrogen or lower alkyl.

[0099]   As defined herein, in some embodiments of Formula II, R$_1$ and R$_2$ taken together with the carbon atoms to which they are attached form an aryl ring. It is preferred that the aryl ring is naphthyl and is especially phenyl.

[00100]   Moreover, in Formula II, as defined herein R$_{14}$ is preferably H or a positively charged electron withdrawing group, as defined hereinabove, or

$$\begin{array}{c} O \\ \| \\ -P-R_{10} \\ | \\ R_{11} \end{array}$$,
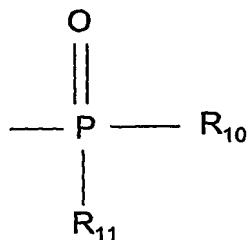
wherein R$_{10}$ and R$_{11}$ are as defined herein and the electron withdrawing group is as defined hereinabove. It is most preferred that R$_{14}$ is

$$\underset{R_{11}}{\overset{\overset{\displaystyle O}{\|}}{-P}} - R_{10}$$
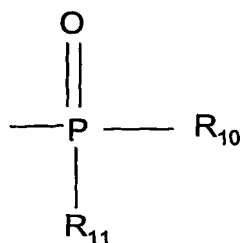
[00101]   The preferred values of R_{10} and R_{11} are as defined hereinabove with respect to Formula I.

[00102]   Preferred structures of Formula II have the formula:

$$\underset{O}{\overset{\displaystyle D-A}{\underset{E}{\overset{}{\bigcirc}}}} \overset{Y_1 \, \diagup\! Q_1}{\underset{G}{\diagdown}} \;\; N - OR_{14}$$
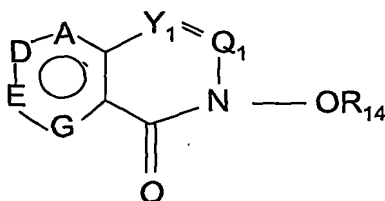
wherein

Y_1 is N or CR_{15};

R_{15} is H or lower alkyl;

Q_1 is N or CR_{16};

R_{14} is hydrogen, a positively charged electron withdrawing group,

R_{16} is H or lower alkyl;

$$R_{10} - P = O \atop \underset{R_{11}}{|} \qquad , \qquad \underset{Rb_1}{\overset{\displaystyle O}{\underset{Rc_1}{\overset{\|}{\underset{}{\bigcirc A_1}}}}} \overset{\displaystyle P}{\underset{T}{}} \overset{Rb_2}{\underset{Rc_2}{\bigcirc B}} ,$$
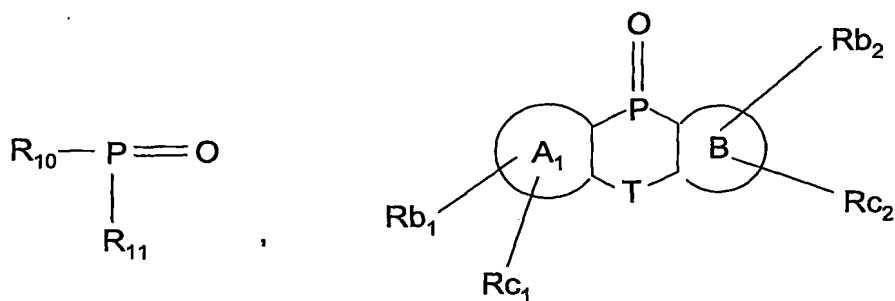
SO_2R_{17}, lower alkyl carbonyl, aryl carbonyl, lower alkyl aryl or BLK_1-AA_1;

AA_1 is an amino acid or peptide less a hydrogen atom on the N-terminus and an OH on the C-terminus;

     BLK_1 is an amino protecting group;

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R_{10} is OR_{12}, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl, or lower cycloalkenyl lower alkyl;

R_{11} is OR_{13}, lower alkyl, aryl, aryl lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl or lower cycloalkenyl lower alkyl;

and R_{10} and R_{11} may optionally be connected by a bridging group selected from the group consisting of O, S, NR_{30}, or $(CHR_{30})_m$, wherein each R_{30} is independently lower alkyl or hydrogen and m is 1-3; and

R_{12} and R_{13} are independently lower alkyl, lower cycloalkyl, lower cycloalkyl lower alkyl, heterocyclic, heterocyclic lower alkyl, lower cycloalkenyl or lower cycloalkenyl lower alkyl;

ring A_1 and ring B are independently aromatic containing 6 to 14 ring carbon atoms or cycloalkenyl or cycloalkyl each containing 5 to 14 ring carbon atoms, and

R_{b1}, R_{c1}, R_{b2}, R_{c2} are independently hydrogen, lower alkyl or electron donating group and T is CHR_{31}, O, S or NR_{31} wherein R_{31} is hydrogen or lower alkyl;

A is N or CR_{24};

D is N or CR_{25};

E is N or CR_{26};

G is CR_{27} or N;

R_{24}, R_{25}, R_{26} and R_{27} are independently hydrogen or lower alkyl or an electron donating group or R_{25} and R_{26} or R_{24} and R_{25} or R_{26} and R_{27} taken together with

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the carbon atoms to which they are attached form an aryl ring, but at least one of

A, D, E and G is N.

[00103]   It is also preferred that structures of Formula II have the formula:

  or
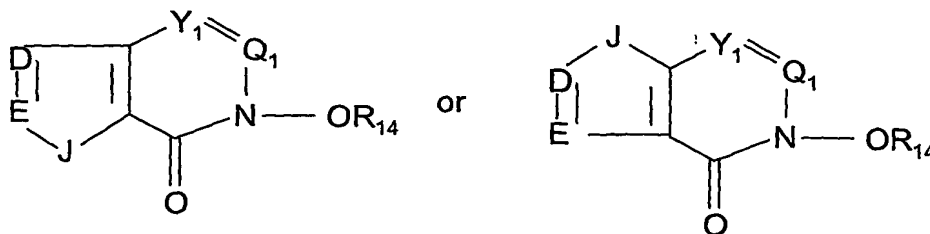
wherein

Y_1, Q_1 and R_{14}, are as defined hereinabove,

D is CR_{25} or N;

G is CR_{26} or N;

J is NR_{28}, O, $CR_{28}R_{29}$ or $S(O)_p$;

R_{25} and R_{26} are independently hydrogen or lower alkyl or an

electron donating group or R_{25} and R_{26} taken together with the carbon atoms to

which they are attached form an aryl group;

R_{28} is hydrogen or electron donating group or lower alkyl;

R_{29} is hydrogen or lower alkyl and

p is 0, 1 or 2.

[00104]   More preferred structures of Formula II have the formula

X

XI

or

XII
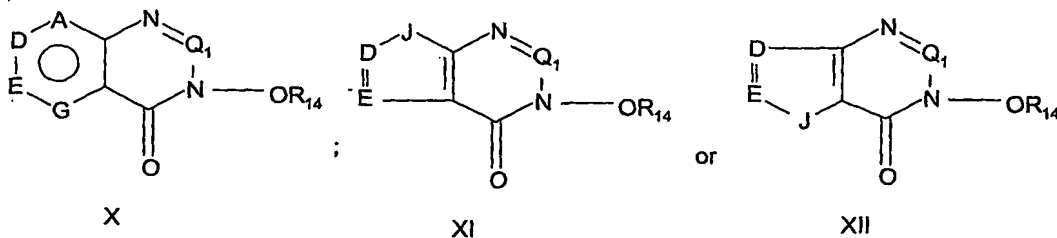
wherein   A is N or CR_{24},

D is CR_{25} or N,

E is CR_{26} or N.

G is CR_{27} or N;

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J is NR_{28}, O, CR_{28}, R_{29} or $S(O)_p$; and

R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, p, Q_1, and R_{14} are as defined hereinabove.

[00105]   With respect to Formula II, and all of its various embodiments depicted hereinabove, it is preferred that no more than two of A, D, E, and G are N. It is most preferred that only one of A, D, E, or G is N. Further, it is preferred that R_{24}, R_{25}, R_{26} or R_{27} are independently hydrogen or an electron donating group, as defined herein. The preferred electron donating groups are lower dialkylamino, especially N, N-dimethylamino and lower alkoxy, e.g., methoxy.

[00106]   Moreover, it is preferred that T is CH_2, O, S or NH and more preferably CH_2 or O. Preferred structures of Formula X have the formulae
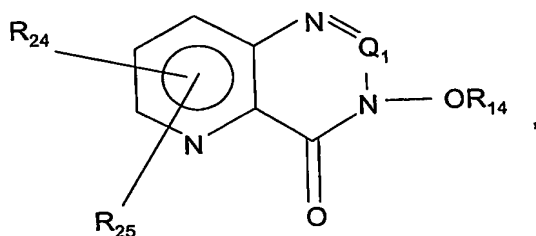
XI
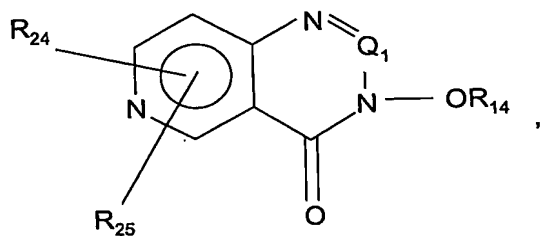
XII
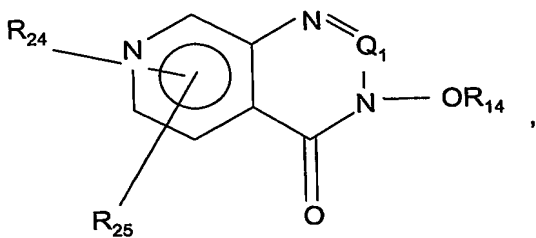
XIII
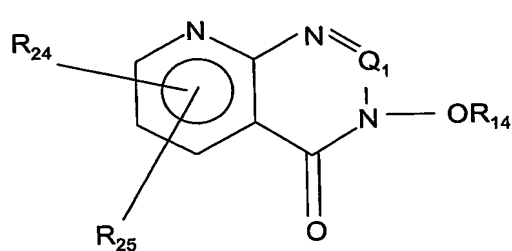
XIV

or N-oxides thereof, wherein R_{24}, R_{25}, Q_1 and R_{14} are as defined hereinabove.

[00107]   Of the structures of Formulae XI-XIV, the most preferred compound is that of XIa viz.

44

Xla
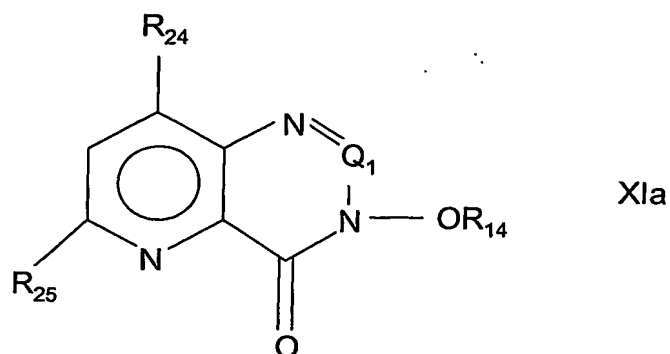
wherein R_{24}, R_{25}, Q_1 and OR_{14} are as defined herein.

[00108]   Preferred structures of Formula II also have the formula

or

XV   XVI
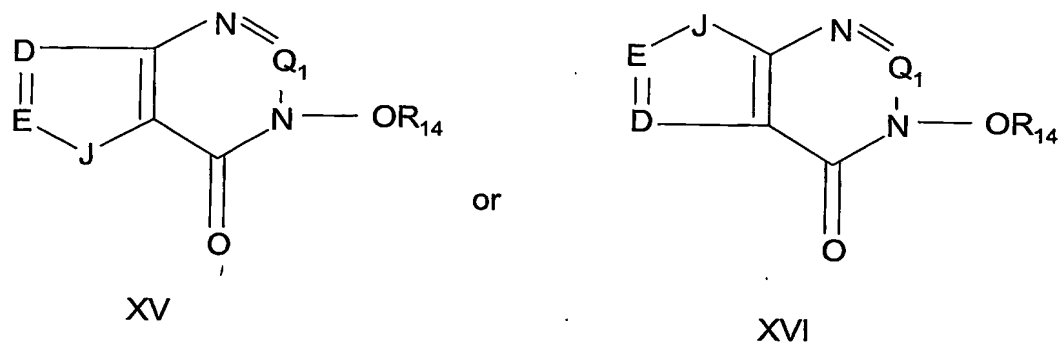
wherein J, E, D, Q_1 and R_{14} are as defined hereinabove. It is preferred that R_{24} is hydrogen, lower alkyl or electron donating group as defined hereinabove and R_{25} is hydrogen or lower alkyl. It is most preferred that R_{25} is hydrogen and it is most preferred that R_{24} is an electron donating group or hydrogen.

[00109]   Preferred values of J are O or S(O)p or NH; the preferred value of p is 1.

[00110]   Preferred structures of Formula II also have the following formula:

45
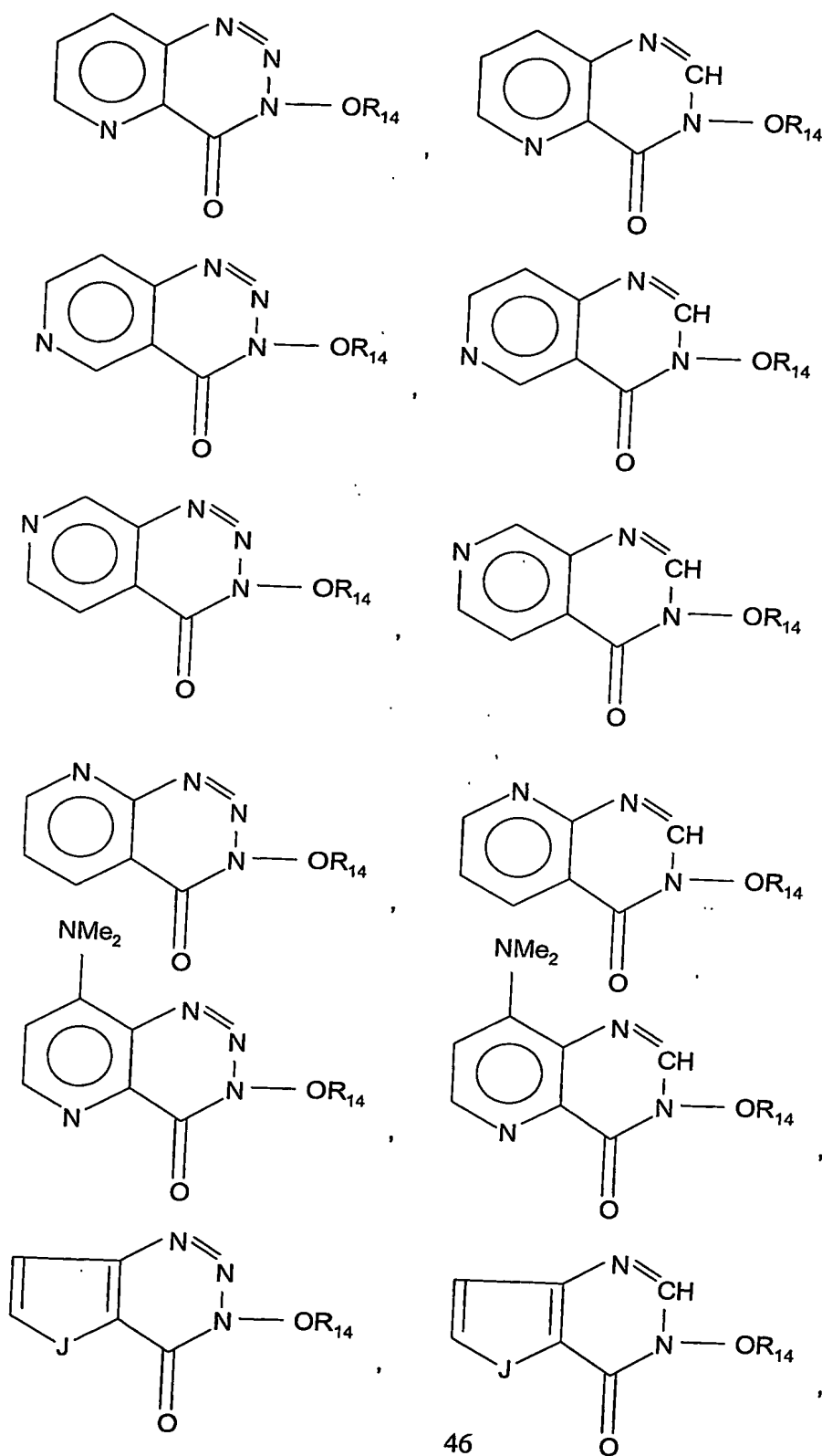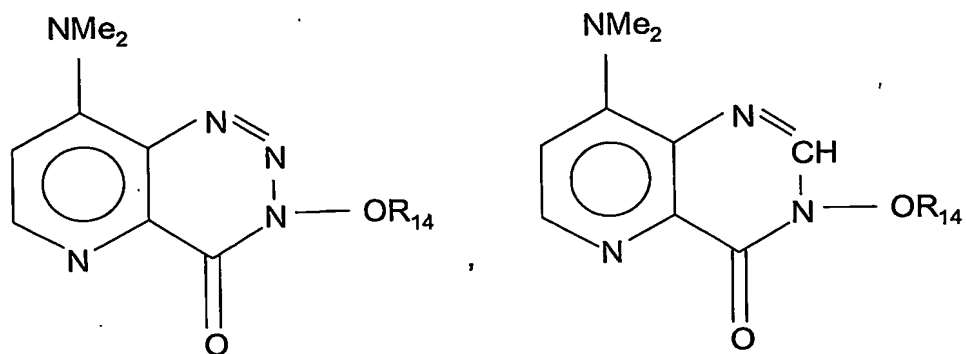
or the N-oxides thereof.

[00111] In the various structures described herein whether it is for compounds of Formula I or II or any other embodiment of the present invention depicted herein, the preferred values of T is O, S, NH or CH_2. In addition, in the various structures depicted hereinabove, it is preferred that m is 1 and that R_{30} is H.

[00112] Compounds of Formula II or salts, especially wherein the cationic portion has the structure of Formula II, are prepared by art recognized techniques. For example compounds of Formula II are prepared by reacting compounds of Formula XVII whenever R_{14} is hydrogen
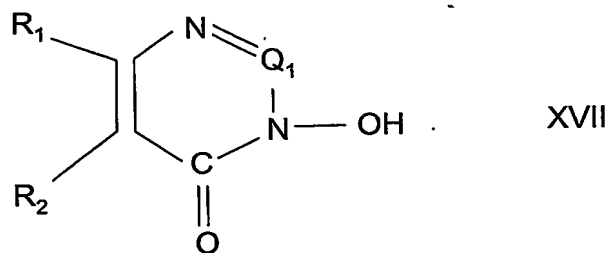
with $R_{14}L$, wherein R_1, R_2, Q_1, and R_{14} is as defined herein and L is a leaving group, such as halo, (e.g. chloro, bromo or iodo). However, if R_{14} is a positively charged electron withdrawing group, then the structures of Formula II is a cation, and in this case, there would be an anion associated with this cationic moiety. For example, when R_{14} is

$$
\begin{array}{c}
O \\
\parallel \\
R_{11}\text{-} P - R_{10}
\end{array}
$$, the compounds of Formula XVII is reacted with
$$
\begin{array}{c}
O \\
\parallel \\
R_{11} - P - R_{10} \\
\backslash \\
L
\end{array}
$$
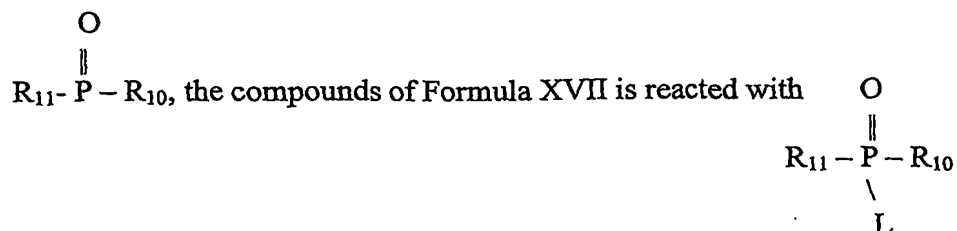
under substitution reaction conditions wherein R_{12} and R_{11} and L are as defined hereinabove. It is preferable that reaction is run in an inert polar organic solvent and that the reactants are soluble therein at room temperature. It is also preferred that the product is insoluble in the solvent at room temperature Examples of the solvent that could be used include chloroform, carbon tetrachloride, ethyl ether, dioxane, tetrahydrofuran, methylene, chloride, and the like. The reaction takes place at effective temperatures, which may range from the melting point of the solvent up to reflux temperatures, but it is preferred that the reaction takes place at about room temperature or at slightly higher temperatures up to the reflux temperature of the solvent. It is especially preferred that the reaction take place at room temperature or at slightly elevated temperatures such as up to 60°C.

[00113]   Compounds of Formula XVII can also be prepared by art recognized techniques known to one of ordinary skill in the art. An exemplary procedure is as follows:

XXIII

XXIV
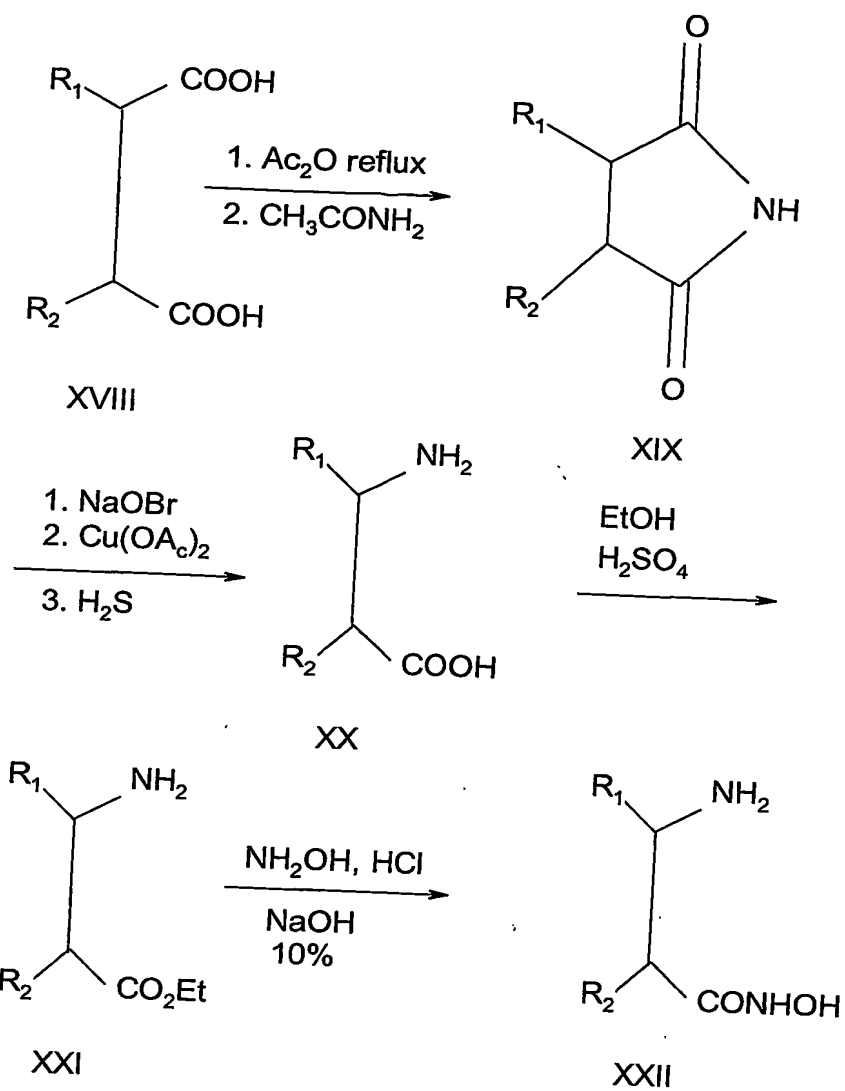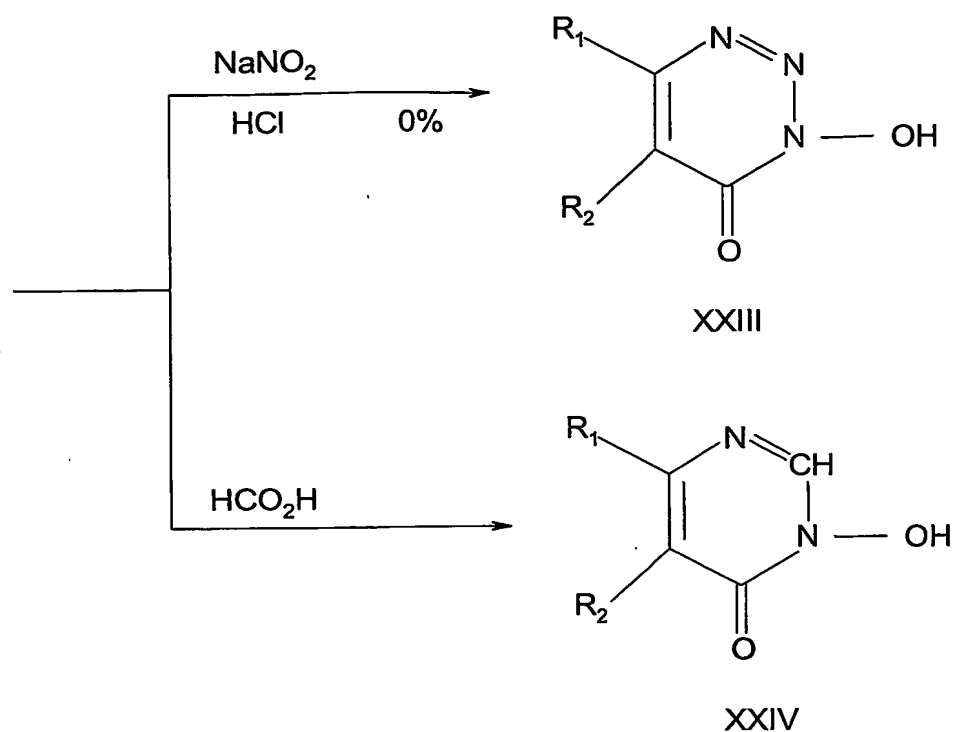
[00114] For example, a compound of Formula VII is refluxed with acetic anhydride to give the corresponding anhydride under anhydride formation conditions. The anhydride product was then treated with acetamide under amide forming conditions to give the corresponding cyclic imide XIX. The cyclic imide XIX is subjected to conditions effective for it to undergo Hoffman rearrangement, e.g., by reacting it with sodium hypobromite (or sodium hydroxide and bromine) followed by hydrolysis. For example, the cyclic imide XIX is reacted with NaOBr, Copper (II) acetate and H_2S to provide the amino carboxylic acid (XX) with one less carbon atom. Esterification of acid XX under the esterfying conditions gives the corresponding ester XXI. Treatment of XXI with hydroxylamine in acid (hydroxyl ammonium salt) under esterification reaction conditions gives the hydroxamic acid derivative XXII. Diazotization followed by intramolecular cyclization gives the azo derivatives XXIII. On the other hand, reaction of the hydroxamic with formic acid under effective conditions, such as by heating the hydroxamic acid with formic acid at effective temperatures e.g., temperatures ranging from just above room temperature up to and including

reflux temperatures, and preferably, at the reflux temperature of formic acid gives the product XXIV.

[00115]   Of course, various combinations and permutations of the formulae described herein are also contemplated by the present invention. In addition, Markush groupings containing less than all of the elements described hereinabove as well as the various permutations thereof are also contemplated by the present invention.

[00116]   As described herein, the compounds or salts or N-oxides described hereinabove are useful in promoting peptide coupling, i.e., the reaction between a free amino group of a first amino acid or first peptide with a free carboxy group or acylating group of a second amino acid or peptide. The process of the present invention is general; it can be used in effecting the coupling of a dipeptide of an amino acid, a tripeptide and an amino acid, dipeptides, pentapeptide, higher peptides, polypeptides, etc.

[00117]   When the compound of Formula I or structures of Formula II reacts with an amino compound such as an amino blocked amino acid or protein of the formula BLK_1-AA_1, the corresponding amino acid ester of the one of the following compounds is formed depending on the identity of the coupling agent:
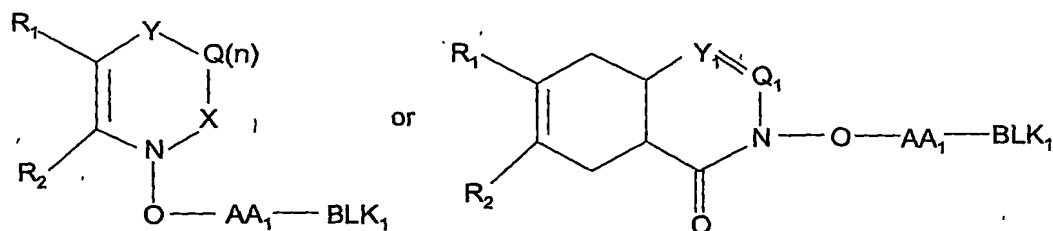
wherein AA_1 is an amino acid or protein as defined herein, BLK_1 is a blocking group as defined herein and Y, Q, Q_1, n, X, R_1, and R_2 are as defined hereinabove. This amino acid ester can then react with a compound having a free amino group such as an arylamino, alkylamino, lower aryl amino, heterocyclic amino,

heterocyclic lower alkylamino, lower cycloalkylamino, lower cycloalkyl lower alkyl amino, and the like designated as $R_{33}R_{34}$ NH, wherein R_{33} and R_{34} are independently hydrogen, lower alkyl, aryl or lower aryl alkyl, to form a compound of the formula:

$$BLK_1 - AA_1 - N\ \overset{R_{33}}{\underset{R_{34}.}{\diagdown}}$$
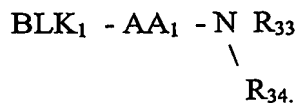
[00118]   Removal of the blocking group by techniques known to one skilled in the art affords the product:

$$AA_1NR_{33}R_{34}.$$

[00119]   This technique is extremely useful when the second amino compound is an amino acid or peptide having a free amine group, designated as AA_2. For example, if the coupling agent is a compound of Formula I, a peptide may be formed between AA_1 and AA_2 as follows, for example,
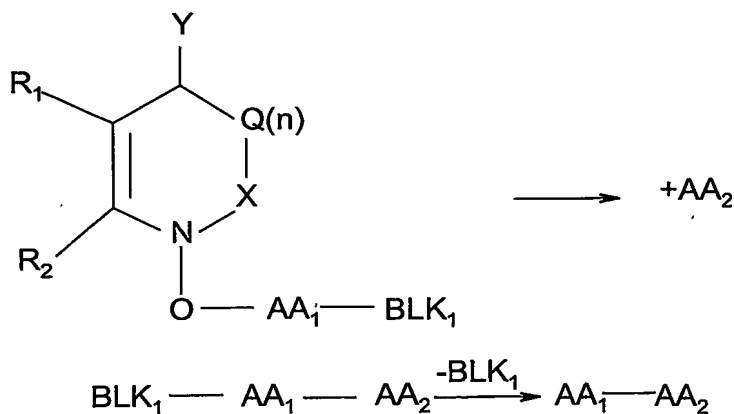
wherein AA_1, AA_2, BLK_1, R_1, R, Y, Q, n and X are as defined herein.

[00120]   If the coupling agent contains a structure of Formula II,

, whether compound or cationic portion of the salt,
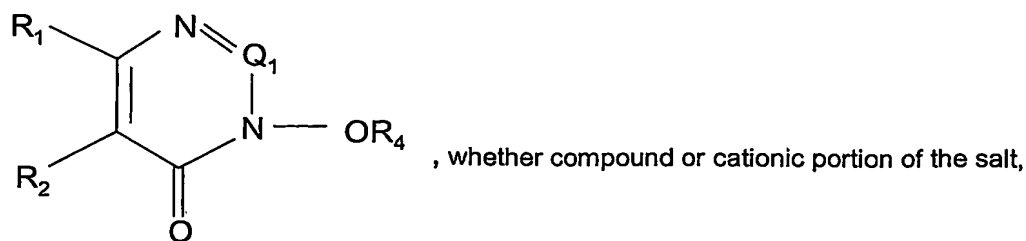
then the reaction becomes
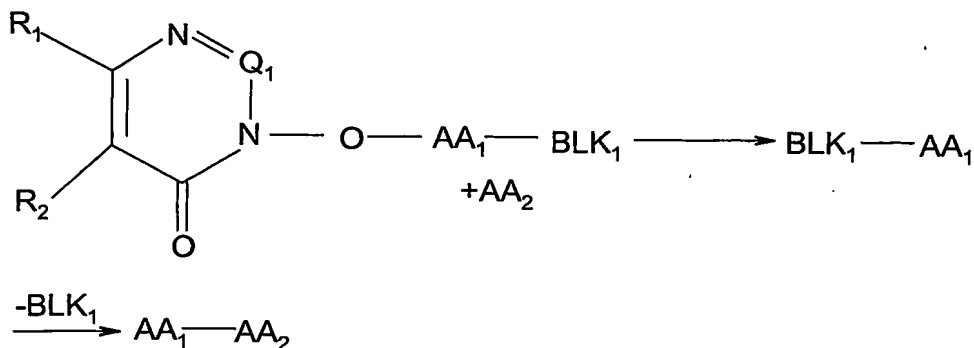
[00121]   In the reaction AA_1, AA_2, BLK_1, R_1, R_2, and Q_1 are as defined herein.

[00122]   The blocking group can be any of the blocking groups known in the art but the preferred blocking groups are FMOC, BOC, benzyloxycarbonyl BSMOC and Bspoc.

[00123]   The term "amino acid" or AA, AA_1, or AA_2 as used herein refers to an organic acid containing both a basic amino group (NH_2) and an acidic carboxyl group. (COOH).

[00124]   Therefore, said molecule is amphoteric and exists in aqueous solution as dipole ions. (See "The Condensed Chemical Dictionary", 10th Ed., edited by Gessner G. Hawley, Van Nostrand Reinhold Company, London, England p. 48 (1981). The preferred amino acids are the α-amino acids. They include but are not limited to the 25 amino acids that have been established as protein constituents. They must contain at least one carboxyl group and one

primary or secondary amino group in the amino acid molecule. The term includes such proteinogenic amino acids as alanine, valine, leucine, isoleucine, norleucine, proline, hydroxyproline, phenylalanine, tryptophan, 2,4-diamino butyric acid, methionine, glycine, serine, threonine, cysteine, cystine, glutamic acid, lysine, hydroxylysine, ornithine, arginine, histidine, penicillamine, naphthylamine, α-phenylglycine, aspartic acid, asparagines, glutamine, arginine, tyrosine, and the like.

[00125]　　As used herein, the term "peptide" refers to the class of compounds composed of amino acid units chemically bound together with amide linkages. A peptide may contain as little as two amino acid residues or may consist of a polymer of amino acid residues (polypeptide).

[00126]　　As used herein, the terms "amino acid" and "peptide" also include amino acids and peptides, respectively containing blocking (protecting) groups. These protecting "groups" block the amino group or the carboxyl group of the amino acid or peptide not involved in or taking part in the coupling in order to prevent unwanted side reactions. These protecting groups also protect reactive groups on the side chain.

[00127]　　A number of blocking reagents for amino groups are known in the art and have been utilized in the syntheses of peptides. These blocking groups are discussed in U.S. Patent Nos. 3,835,175, 4,508,657, 3,839,396, 4,581,167, 4,394,519, 4,460,501 and 4,108,846, the contents of all of which are incorporated by reference as if fully set forth herein. Other amino protecting groups are discussed in U.S. Patent Nos. 5,221,754, 5,510,491 and 5,637,719 the contents of which are also incorporated by reference. Other amino protecting groups are described in an article entitled "Solid Phase Peptide Synthesis", by G. Barany and R.B. Merrifield in THE PEPTIDES, Vol. 2, edited by E. Gross and J. Meienhoffer, Academic Press, N.Y., N.Y. 100-118 (1980), and in the book entitled "PROTECTIVE GROUPS IN ORGANIC SYNTHESIS" by T.W. Green,

John Wiley & Sons, New York, the contents of all of which are being
incorporated by reference.

[00128]   The term amino acid protecting group, (BLK, BLK$_1$)as used
herein, refers to blocking groups which are known in the art and which have been
utilized to block the amino NH$_2$) group of the amino acid. Blocking groups such
as 9-lower alkyl-9-fluorenyloxycarbony, 2-chloro-1-indanylmethoxy-carbonyl
(CLIMOC) and benz [f] indene-3-methyloxycarbonyl (BIMOC) and dbd-TMOC
are discussed in U.S. Patent Nos. 3,835,175, 4,508,657, 3,839,396, 4,581,167,
4,394,519, 4,460,501 and 4,108,846 referred to hereinabove, the contents of
which are incorporated by reference. Moreover, other amino protecting groups
such as 2-(t-butyl sulfonyl)-2-propenyloxycarbonyl (Bspoc) and benzothiophene
sulfone-2-methoxycarbonyl (Bsmoc). Other N-amino protecting groups include
such groups as the t-butyloxycarbonyl (BOC), t-amyloxycarbonyl (Aoc), β-
trimethylsilyl-ethyloxycarbonyl(TEOC), adamantyl-oxycarbonyl (Adoc), 1-
methyl-cyclobutyloxycarbonyl (Mcb), 2-(p-biphenylyl)propyl-2-oxycarbonyl
(Bpoc), 2-(p-phenylazophenyl)propyl-2- oxycarbonyl (Azoc), 2,2-dimethyl-3,5-
dimethyloxybenzyloxycarbonyl (Ddz), 2-phenylpropyl-2-oxycarbonyl (Poc),
benzyloxycarbonyl (Cbz), p-toluenesulfonyl aminocarbonyl (Tac), o-
nitrophenylsulfenyl (Nps), dithiasuccinoyl (Dts), Phthaloyl, piperidine-
oxycarbonyl, formyl, trifluoroacetyl and the like.

[00129]   These protecting groups can be placed into four categories:
      1) a base labile Nα-amino acid protecting group such as FMOC, and the
      like.
      2) protecting groups removed by acid, such as Boc, TEOC, Aoc, Adoc,
      Mcb, Bpoc, Azoc, Ddz, Poc, Cbz, 2-furanmethyloxycarbonyl (Foc), p-
      methoxybenzyloxycarbonyl (Moz), Nps, and the like.
      3) protecting groups removed by hydrogenation such as Dts, Cbz.
      4) protecting groups removed by nucleophiles, such as Bspoc, Bsmoc

and Nps and the like.

5)   protecting groups derived from carboxylic acids, such as formyl, acetyl, trifluoroacetyl and the like, which are removed by acid, base or nucleophiles.

[00130]   A variety of carboxy protecting groups known in the art may be employed. Examples of many of these possible groups may be found in "Protective Groups in Organic Synthesis", by T.W. Green, John Wiley & Sons, 1981, the contents of which are incorporated by reference. These examples include such groups as methyl ester, t-butyl ester, β-trimethylsilylethyl ester, benzyl ester and the like.

[00131]   In addition, during the course of protein synthesis, it may be necessary to protect certain side chains of the amino acids to prevent unwanted side reactions. The various protecting groups are discussed in U.S. Patent No. 5,360,920, the contents of which are incorporated herein by reference.

[00132]   The term "acylating group of an amino acid or peptide" refers to a group on the free carboxy end of the amino acid or peptide that facilitates the acylation reaction, i.e., nucleophilic substitution at the acyl carbon. Examples include the free acid, acid halide, esters, such as lower alkyl esters, phenoxy esters which are unsubstituted or substituted with 1-5 electron withdrawing groups as defined herein; or an anhydride and the like. The preferred acylating derivative is the acid, acid halide, especially the acid chloride or fluoride, and the phenoxy ester.

[00133]   The preferred acylating amino acid is an amino acid group of the formula

BLK-AA-M,
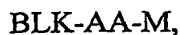
wherein BLK is an amino protecting group

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AA is an amino acid less the H on the COOH moiety and
M is halo or

(R$_a$)$_b$
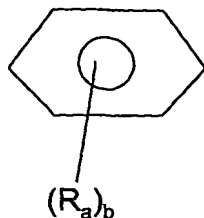
wherein Ra is independently halo, lower alkyl, nitro, cyano or other electron
withdrawing group and b is 0-5. When b is 0, the phenyl group is unsubstituted.

[00134]   The most preferred acylating group of an amino acid is the amino
acid chloride or fluoride. The preparation and use of amino acid chlorides as an
acylating derivative is discussed in an article by Carpino, et al. in J. Org. Chem.,
1986, 51, 3734-3736, the contents of which are incorporated herein by reference.
Briefly, amino acid chlorides can be prepared by reacting the amino acid with
thionyl chloride and recrystallizing the product from a recrystallization reagent,
such as CH$_2$Cl$_2$—hexane.

[00135]   The preparation and use of amino acid fluorides in peptide
synthesis are discussed U.S. Patent No. 5,360,920, the contents of which are
incorporated herein by reference. As described therein, the amino acid fluorides
can be prepared by reacting an N-protected amino acid with the reagent cyanuric
fluoride. This reaction can be run at temperatures as low as 0^0C and up to the
refluxing temperature of the solvent, but it is preferred that the reaction is run at
room temperature. It can also be run in an inert solvent, such as pyridine/CH$_2$Cl$_2$
and the like. The cyanuric fluoride can be prepared from the corresponding
chloride in the presence of potassium fluoride at elevated temperatures ranging
from 150^0 to 250^0C, according to the following equation
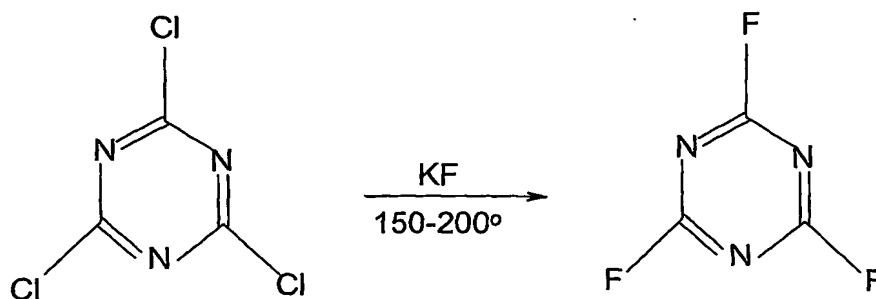
[00136]   A typical preparation of the peptide in accordance with the present invention involves the following steps

1) protection of the free carboxyl group in a first amino acid or a first peptide, unless the amino acid or peptide is anchored to a solid support.

2) protection of the free amino group of a second amino acid or peptide.

3) protection of the side chains, if necessary.

4) coupling the first amino acid or peptide with the second amino acid or peptide in the presence of compounds of Formula I.

5) removal of the protecting groups.

[00137]   The procedure of steps 1-3 can be performed in any order.

[00138]   In the coupling step, the compounds of Formula I or II or salts or N-oxides thereof or cation of Formula II is present in effective amounts. Usually, the first amino acid or peptide is present in approximately equimolar amounts with the second amino acid or peptide. Furthermore, the amount of the compound having Formula I or II used depends upon the amount of peptide or amino acid which is present in the least amount (i.e. the limiting reagent); thus the molar ratio of the compound of Formula I or II to the amino acid or peptide present in the least molar amount, ranges from about 1:3 to about 3:1, although it is preferred that approximately equimolar amounts of the compound of Formula I or II (or salt or N-oxide thereof or cation of Formula II) the first amino acid or peptide and the second amino acid or peptide be used.

[00139]   The coupling reaction usually takes place in an inert organic

solvent such as dimethylformamide (DMF) or ethers, such as ethyl ether, THF or

dioxane. In fact DMF is the preferred solvent in the solid phase synthesis because

of its favorable solvation properties. The reaction takes place under mild

conditions usually ranging from about $0°C$ to about $30°C$. After the peptide is

formed, the blocking groups are removed by techniques known to one skilled in

the art.

[00140]   The following sequence is illustrative of the coupling reaction; in

the examples below, amino acids (AA) are used, although the procedure is general

for amino acids and/or peptides:

$$H - AA_2 - P + BLK - AA_1 - OH \xrightarrow{\text{Compound of Formula I or II or salt or or N-oxide or cation of Formula II}}$$

$$BLK - AA_1 - AA_2 - P - \xrightarrow{-BLK} \xrightarrow{\substack{BLK-AA_3-OH \\ \text{In the presence of Compound of Formula I or II or salt or N-oxide or cation of Formula II}}}$$

$$BLK - AA_3 - AA_1 - AA_2 - P \xrightarrow{-BLK} \xrightarrow{-P} AA_3 - AA_1 - AA_2$$
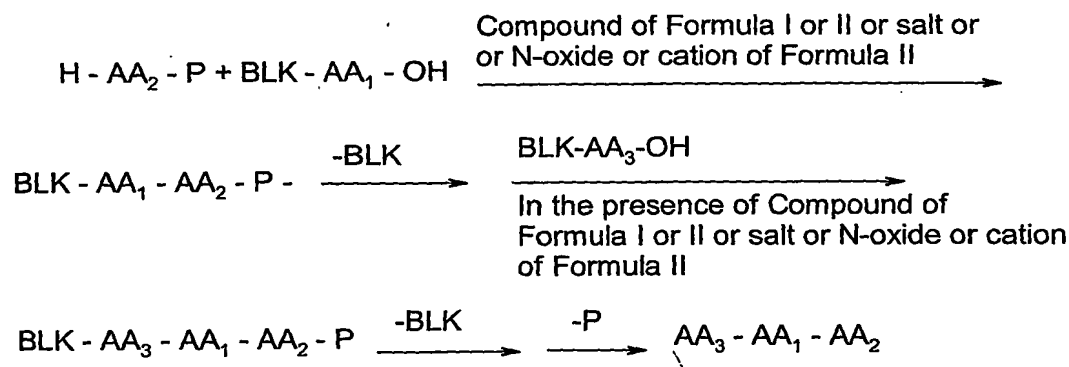
[00141]   In the above scheme, BLK is an amino acid blocking group, AA_1,

AA_2 and AA_3 are first, second and third amino acid respectively and P is a

carboxy protecting group.

[00142]   As shown by the above scheme, the N-α amino protected amino

acid is reacted with a second amino acid in which the carboxy group is protected.

[00143]   A peptide is formed between the first amino acid and the second

amino acid. The peptide chain can be increased by removing the alpha amino

protecting group by techniques known to one skilled in the art and then reacting the corresponding dipeptide with another N-α amino protected amino acid in the presence of a compound of Formula I to form the corresponding tri-peptide. The N-α amino protecting group of the tri-peptide is removed and the above cycle is repeated until the desired peptide has been obtained.

[00144] The present invention can readily be utilized in solid phase peptide synthesis. Solid phase peptide synthesis is based on the stepwise assembly of a peptide chain while it is attached at one end to a solid support or solid phase peptide resin. Two methods are generally well known in the art.

[00145] One, the Merrifield method, employs a solid support for attachment of the amino acid or peptide residues. This method employs N-protected amino acids as building blocks which are added to an amino acid or peptide residue attached to the solid support at the acyl (acid) end of the molecule. After the peptide bond has been formed, the protecting group is removed and the cycle repeated. When a peptide having the desired sequence has been synthesized, it is then removed from the support.

[00146] The second method, the inverse Merrifield method, employs reagents attached to solid supports in a series of columns. The amino acid or peptide residue is passed through these columns in a series to form the desired amino acid sequence.

[00147] These methods are well known in the art as discussed in U.S. Patent Nos. 4,108,846, 3,839,396, 3,835,175, 4,508,657, 4,623,484, 4,575,541, 4,581,167, 4,394,519 as well as in Advances in Enzymology, 32, 221 (1969) and in PEPTIDES, Vol. 2, edited by Erhard Gross and Johannes Meienhoffer, Academic Press, New York pp. 3-255 (1980) and the contents thereof are incorporated herein by reference as if fully set forth herein.

[00148] The compounds of the present invention are useful as coupling agents or bases in peptide coupling. However, their effectiveness is also a function of the solvent system which is used to dissolve the reactants and bases used in the coupling reaction. If the the compound of the present invention is not too soluble in the solvent used in the coupling method, then it should be converted to a form more soluble in the solvent or the solvent should be changed to one in which it is soluble. This is usually not a problem in peptide coupling conducted in solution. But, with some peptide synthesizers, the option of changing the solvent is not available. For example, with some synthesizers, DMF or N-methylpyrrolidone is the solvent utilized. However, compounds of the present invention may not be too soluble in the solvent utilized, such as DMF or N-methylpyrrolidone. Thus, to overcome this problem the compound of the present invention is converted to a compound which is soluble in the solvent utilized, sich as DMF or N-methylpyrrolidone. For example, if the coupling agent or base of the present invention is that of Formula I and if R_{10} or R_{11} is aryl or heteroaryl or if A_1 or B is aryl or heteroaryl, then the trick to overcome this problem is to place a t-butyl or t-amyl group or any other group wherein the carbon atom attached to the aryl or heteroaryl ring is a tertiary carbon.

[00149] If R_{10} or R_{11} or A_1 or B contain more than one ring it is preferred that the t-butyl or amyl group or other tertiary carbon group, such as a tertiary hydrocarbyl group (containing only carbon and hydrogen atoms) is attached to the ring attached to the phosphorus atom. If more than one such group is attached to the rings, it is preferred that these groups are the same.

[00150] The following examples further illustrate the present invention.

[00151] In the examples, the following abbreviations are utilized:
HOAt   1-hydroxy-7-azabenzotriazole

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DIEA	diisopropylethylamine
TEA	triethylamine
CBZ or Cbz	benzyloxycarbonyl
EDC	N-ethyl-N'-[3-dimethylaminopropyl] carbodiimide hydrochloride
Aib	aminoisobutyric acid
THF	tetrahydrofuran
DMF	dimethylformamide
TCFH	1,1,3,3-tetramethylchloroformamidinium hexafluorophosphate
PyClu	1,1,3,3-bis(tetramethylene)chlorouronium hexafluorophosphate
PyBrop	tris(pyrrolindino)bromophosphonium hexafluorophosphate
TFA	trifluoroacetic acid
TMP	2, 4, 6-trimethylpyridine
PCA	p-chloroaniline
DPOPOAt	diphenoxyphosphoryloxy-7-azabenzotriazole
HDATU	O-(3,4-dihydro-4-oxo-5-azabenzo-1,3-diazin-3-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate
N-HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-b]pyridin hexafluorophosphate 3-oxide
O-Dhad	3-oxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine
O-HATU	N-[(1H-1,2,3-triazolo[4,5-b]pyridin-1-yloxy) (dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate
HDTU	O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium hnexaflurorphosphate
HODhat	3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine
HODhbt	3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine
DEPOAt	diethoxyphosphoryloxy-7-azabenzotriazole
DtpOAt	di(o-tolyl)phosphinyloxy-7-azabenzotriazole
HODhad	3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,3-diazine
Odhad	3-oxy-4-oxo-3,4-dihydro-5-azabenzo-1,3-diazine

HDADU	O-(3,4-dihydro-4-oxo-5-azabenzo-1,3-diazin-3-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HDAPyU	O-(3,4-dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-bis(tetramethylene)uronium hexafluorophosphate
PyDAOP	[(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)oxy]tris(pyrrolidino)phosphonium Hexafluorophosphate
DtpOBt	di(o-tolyl)phosphinyloxybenzotriazole
DtpODhbt	3-[di-o-tolyl)phosphinyloxy]-3,4-dihydro-4-oxo-2,3 benzotriazine
DEPDBt	3-(diethoxyphosphoryloxy)-3,4-dihydro-4-oxo-1,2,3-benzotriazine
DPOPDBt	3-(diphenoxyphosphoryloxy)-3,4-dihydro-4-oxo-1,2,3-benzotriazine
DPOPOAt	diphenoxyphosphoryloxy-7-azabenzotriazole
PyDOP	[(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)oxy]tris(pyrrolidino)-phosphonium hexafluorophosophate
HDPyU	O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
DIC	diisopropylcarbodiimide
DCHMA	N,N-dicyclohoxylmethylamine
DmppOAt	2,8-dimethyl phenoxaphosphinyloxy-7-azabenzotriazole
Bt	benzotriazole
Pfp	pentafluorophenyl ester
N-HBTU	1-[bis(dimethylamino)methylene]-1H- benzotriazolium hexafluorophosphate-3-oxide
O-HBTU	N-[1H-benzotriazol-1-yloxy)(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate

EXAMPLE 1

Diethoxyphosphoryloxy-7-azabenzotriazole

(DEPOAt)

[00152]   A solution of HOAt (1.36g, 10 mmol) and triethylamine (TEA) (1.39 mL, 10 mmol) in 15 ml of dry benzene was cooled in an ice bath. To the solution was added dropwise with stirring a solution of diethyl chlorophosphate (1.72 g, 1.45 ml, 10 mmol) in 10 ml of dry benzene under N_2. The addition was completed in 10 min, and stirring was continued at ice-bath temperature for 1 hour and then at room temperature for an additional 4 hours. The reaction mixture was cooled to 5-10°C and TEA HCl was removed quickly by filtration. The colorless clear solution was evaporated to dryness while the temperature was kept below 35°C. Dry hexane was added to the oily residue and the whole was tightly capped under N_2, and placed in a refrigerator (-20°C) for 2 hours. The oily residue solidified as a white solid, which was then recrystallized from dry CH_2Cl_2-hexane to give 1.74 g (63%) of the ester as colorless plates: mp 48-50°C; ^1H NMR ($CDCl_3$): δ 8.77 (dd, 1H), 8.40 (dd, 1H), 7.44(dd, 1H), 4.57 (m, 4H, 2CH_2), 1.47 (2t, 2CH_3); IR (film on NaCl plate): 2987(m), 1596(m), 1395(m), 1306(s), 1026(vs), 838(m), 775(s), 699(m) cm^{-1}.

Anal. Calcd for $C_9H_{13}N_4O_4P$: C, 39.71; H, 4.81; N, 20.58.

Found: C, 39.84; H, 4.72; N, 20.54.

EXAMPLE 2

Di(*o*-tolyl)phosphinyloxy-7-azabenzotriazole

(DtpOAt)

[00153]   A solution of HOAt (0.136 g, 1 mmol) and DIEA (0.21 ml, 1.2 mmol) in 10 ml of dry CH_2Cl_2 was cooled in an ice bath and 0.2647 g (1 mmol) of di(*o*-tolyl)phosphinoyl chloride (DTP-Cl) was added to the solution portion wise with stirring under N_2. The stirring was continued in the ice-bath for 30 minutes and then at room temperature for 5 hours. The resulting colorless solution was washed with cold saturated $NaHCO_3$ solution (2 x 10 ml) and brine (2 x 10 ml), and dried over $MgSO_4$ to give 0.26 g (69%) of white solid after removing the solvent. Recrystallization from CH_2Cl_2-hexane gave 0.21 g of an analytically pure sample of the ester as colorless block crystals: mp 170-172°C; ^1H NMR ($CDCl_3$): δ 8.74 (dd, 1H), 8.32 (d, 1H), 7.99 (dd, 1H), 7.56 (m, 2H, Ar-H), 7.35 (m, 6H, Ar-H), 2.70 (d, 6H, 2CH₃); IR (KBr): 3064(w), 1592(s), 1458(m), 1379(m), 1334(m), 1238(sh, s), 1113(s), 928(m), 811(s), 770(s), 691(s) cm^{-1}.

Anal. Calcd for $C_{19}H_{17}N_4O_2P$: C, 62.62; H, 4.70; N, 15.37.

Found: C, 62.23; H, 4.72; N, 15.47.

EXAMPLE 3

Synthesis of 3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine

(HODhat)

[00154]   **A. Quinolinimide.** 2,3-Pyridinedicarboxylic acid (22.5 g, 0.135 mol) was added to 25 ml of acetic anhydride and the mixture was heated with stirring to reflux on a steam-bath. A clear colorless solution was obtained after about 1 hour, and the heating was continued for 2 more hours. Then, acetic acid and excess acetic anhydride were distilled off (~ 12.6 g) until the temperature of the residual solution reached 165°C. The residue which remained in the flask solidified to a dust red solid upon cooling to room temperature.

[00155]   To the above solid, acetamide (14 g, 0.23 7 mol, 1.75 equiv) was added and the mixture was heated overnight by means of an oil-bath, keeping the temperature at 120-125°C. After cooling to room temperature, the solid was collected and washed with acetic acid (2 x 10 ml) and water (3 x 50 ml). The gray solid was suspended in 250 ml of hot water and the mixture stirred for 15 min to give by filtration 15 g (75%) of the above-identified imide as a gray solid: mp237° - 239°C (lit. mp 233°C).

[00156]   **B. 3-Aminopicolinic Acid.** Quinolinimide (50 g, 0.344 mol) was dissolved in 1000 ml of 10% NaOH while cooling in an ice-bath and to the solution was added slowly with stirring and cooling a cold NaOBr solution, which had been obtained by mixing 56 g (18 ml, 0.344 mmol) of Br_2 with 350 ml of 15% NaOH in an ice-bath. After the addition had been completed, stirring was continued in the ice-bath for 15 min and at room temperature for 1 hour. The resulting mixture was then heated to 85°C with stirring for 1 hour. After cooling, the mixture was neutralized to pH 5-6 by means of 50% H_2SO_4 and the whole was kept at room temperature overnight.

[00157]   The resulting white cloudy mixture was heated and the clear solution treated with a hot solution of 22 g (0.11 mol) of copper (II) acetate monohydrate in 400 ml of H_2O and 10 ml of acetic acid. The mixture was heated with a steam bath for 15 min and then cooled at room temperature and the copper salt collected and washed twice with cold water.

[00158]   The copper salt was re-suspended in 500 ml of water, and H_2S was passed through the suspension for 2 to 3 hours while stirring. Black CuS was removed from the mixture by filtration and the filtrate decolorized with charcoal. Removal of water gave a dust yellow solid, which was recrystallized form water-ethanol (1:1 v/v) to give 29 g (60%) of the above-identified acid as a cream-yellow solid: mp 212-214°C (lit. mp 210°C).

[00159]   **C. Ethyl 3-Aminopicolinate.** A mixture of 3-aminopicolinic acid (5.07 g, 36 mmol), absolute ethanol (20 ml) and concentrated H_2SO_4 (6 ml) was refluxed for 48 hours. After cooling, the mixture was concentrated to about 15 ml and poured into 15 g of ice. The mixture was basified with concentrated aqueous ammonia to pH 8-9 with cooling in an ice bath, and the white precipitate that separated was collected by filtration. The filtrate was extracted with ether (4 x 50 ml), and the ether layer washed with brine (4 x 50 ml) and dried over $MgSO_4$. Evaporation of the ether solution afforded another solid. Both fractions were combined and recrystallized from benzene-hexane to give 4.05 g (68%) of the above-identified ethyl ester as white needles: mp 126-127.5°C (lit mp 131-133°C, yield 42%); ^1H NMR ($CDCl_3$): δ8.09 (dd, 1H), 7.23 (dd, 1H), 7.04 (dd, 1H), 5.76 (s, 2H, NH_2), 4.46 (q, 2H, CH_2), 1.45(t, 3H, CH_3).

[00160]   **D. 3-Amino-2-picolinehydroxamic Acid.** Hydroxylamine hydrochloride (16.3 g, 0.233 mol) was added slowly with stirring and cooling to 110 ml of aqueous NaOH solution prepared from 18.7 g (0.467 mol) of NaOH. To the solution, 19.4 g (0.116 mol) of the ethyl ester prepared in Section C

hereinabove was added portion wise followed by 110 ml of methanol, and the mixture was stirred at room temperature for 48 hours. The solution was concentrated under reduced pressure to about 100 ml and neutralized with cooling to pH 5-6 with 25% HCl. A white precipitate that separated was collected by filtration, washed with a small amount of cold water, and dried over P_2O_5 in vacuo to give 17.8 g (100%) of the 3-amino-2-picoline-hydroxamic acid as a white solid, which was pure enough for the next step. An analytical sample of this acid was obtained in 90% yield after two recrystallizations from MeNO$_2$-MeOH-EtOAc as white block-like crystals: mp 131-133°C (lit. mp 143-145°C, yield 49%); ^{1}H NMR (DMSO-d$_6$CDCl$_3$): δ10.91 (s, 1H, OH), 8.89 (s, 1H, NH), 7.74 (t, 1H), 7.16 (d, 2H), 6.71 (s, 2H, NH$_2$); IR (KBr): 3443(m), 3334(s), 1660(s, CON), 1606(s), 1262(w), 1017(w), 805(m) cm^1.

[00161] **E. 3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine (HODhat).** To a suspension of finely powdered product produced in step D hereinabove (7.3 g, 47.6 mmol) in 28 ml of water, 8.5 ml of concentrated HCl was added while stirring. While cooling in an ice-bath, a cold solution of NaNO$_2$ (4.93 g, 71.4 mmol) in 5 ml of water was added dropwise to the mixture and the temperature was maintained below 5°C. After completion of the addition, stirring in the ice-bath was continued for another 30 minutes, and the solid which had precipitated was collected by filtration, washed with a small amount of cold water and air dried to give 2.52 g (32%) of the triazine as a yellow solid: mp 195°C (explodes) [lit mp:195°C (explodes)]. The analytical sample (2.1 g) was obtained by recrystallization from EtOHwater (9:1 v/v) as light orange-yellow needles: mp 203°C (explodes); ^{1}H NMR (DMSOd$_6$): δ9.13 (dd, 11H), 8.65 (dd, 111), 8.08 (dd, 11H); IR (KBr): 2600(broad, OH), 1713(vs, C(O)N), 1574(s), 1420(m), 1230(sh, s), 1185(s), 1066(sh, s), 974(sh, m), 794(m) cm^{-1}.

[00162] 3-Hydroxypicolinic acid (1.6 g, 20%) was also isolated from the mother liquor as light pink needles: mp 222-224°C (lit. mp 220-222°C). Anal. Calcd for $C_6H_5NO_3$: C 51.80, H 3.62, N 10.07; Found: C 51.52, H3.58, N 9.98.

EXAMPLE 4

3-Hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,3-diazine (HODhad)

[00163]   A mixture of 1.224 g (8 mmol) of the product produced in Step D
of Example 3 and 3 ml of 98% formic acid were heated under reflux for 15
minutes after which 8 ml of water was added, and the whole was boiled for 15
minutes and cooled to room temperature. The precipitate that separated was
collected by filtration and washed with water (2 x 5 ml). After being boiled with
ethanol twice, there was obtained 0.71 g (55%) of HODhad, which was obtained
in an analytically pure form as a yellow solid: mp 318.5-320^{0}C (dec.); ^{1}H NMR
(DMSO-d$_6$): δ12.15 (br, 1H, OH), 8.85 (dd, 1H), 8.69 (s, 1H, CH), 8.17 (dd, 1H),
7.84 (dd, 1H); IR (KBr): 2625(broad, OH), 1683(sh, vs, CON), 1600(w),
1446(m), 1410(s), 1359(s), 1223(s), 990(s), 902(m), 791(s) cm^{-1}.

Anal. Calcd for C$_7$H$_5$N$_3$O$_2$: C, 51.53; H, 3.09; N, 25.76.

Found: C, 51.46; H, 3.00; N, 25.80.

EXAMPLE 5

CBZ-Aib-ODhat

[00164]   In an ice bath, 0.3168 g of EDC·HCl (1.65 mmol) was added with stirring to a suspension of CBZ-Aib-OH (0.3 555g, 1.5 mmol) and HODhat (the product of Ex. 4) (0.246 g, 1.5 mmol) in 10 ml of THF and 5 ml of DMF. The resulting mixture was stirred at room temperature for 3 hours. Solvents were removed _in vacuo_ and the oily residue was extracted with 40 ml of EtOAc. The EtOAc solution was washed with 5% aqueous citric acid (3 x 10 ml), 10% NaHCO$_3$ solution (3 x 10 ml) and brine (3 x 10 ml), and dried over MgSO$_4$.

[00165]   Evaporation of solvent gave a cream yellow oily residue, which solidified after drying in vacuo over P$_2$O$_5$ overnight. The crude solid was purified by flash chromatography with EtOAc as eluent to give 0.46 g (80%) of the above-identified ester as a cream yellow solid: mp 57-59°C; ^{1}H NMR (CDCl$_3$): δ 9.05 (dd, IH), 8.49 (dd, IH), 7.86 (dd, IH), 7.26 (m, 5H, C$_6$H$_5$), 5.47 (s, IH, NH), 5.10 (s, 2H, CH$_2$), 1.73 (s, 6H, 2CH$_3$); IR(KBr): 1813(m, COO), 1735(vs, NCO), 1267(s), 1052(s) cm^{-1}.

Anal. Calcd for C$_{18}$H$_{17}$N$_3$O$_5$: C, 56.39; H, 4.47; N, 18.27.

Found C, 56.10; H, 4.56; N, 18.08.

EXAMPLE 6

CBZ-Aib-ODhad.

[00166]   As described for EXAMPLE 6, 0.2447g (1.5 mmol) of HODhad (the product of Ex. 5) was treated with 0.3555g (1.5 mmol) of CBZ-Aib-OH in the presence of EDC HCl in 15 ml of a THF-DMF mixture (2:1) to give 0.52 g (90%) of the above product as a white solid: mp 48-50°C; ^{1}H NMR(CDCl$_3$): δ 8.89 (dd, 1H), 8.13 (dd, 1H), 7.92 (s, 1H, CH), 7.71 (dd, 1H), 7.36 (m, 5H, C$_6$H$_5$), 5.47 (s, 1H, NH), 5.15 (s, 2H, CH$_2$), 1.75 (s, 6H, 2CH$_3$); IR. (KBr): 1809(s, COO), 1718(vs, NCO), 1590(sh, m), 1522(sh, m), 1468(m), 1274(sh, s), 1059(s), 974(w) cm^{-1}.

Anal. Calcd for C$_{19}$H$_{18}$N$_4$O$_5$: C, 59.68; H, 4.74; N, 14.65.

Found: C, 59.43; H, 4.80; N, 14.58.

EXAMPLE 7

Me₃C-COODhat
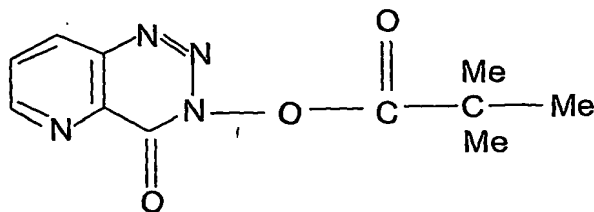
[00167]

[00168]   Under an atmosphere of dry N_2, TEA (0.42 ml, 3 mmol) was added to a suspension of HODhat (0.3282 g, 2 mmol) in 10 ml of dry methylene chloride. The resulting mixture was cooled to 0°C and a solution of pivaloyl chloride (0.27 ml, 2.2 mmol) in 5 ml of dry methylene chloride was introduced dropwise with stirring. The stirring was continued for 30 minutes in an ice-bath, and the temperature was allowed to rise to room temperature. After 4 hours, the mixture was diluted with 30 ml of CH_2Cl_2 and the whole washed with saturated $NaHCO_3$ (3 x 20 ml), brine (2 x 20 ml), and water (2 x 20 ml), and finally dried over anhydrous $MgSO_4$. Evaporation of solvent gave a pale yellow solid, which was recrystallized from EtOAc-hexane to give 0.31 g (6 1%) of the analytically pure ester product as colorless needles: mp 137-139°C; ^{1}H NMR (CDCl₃): δ 9.16 (dd, 1H), 8.57 (dd, 1H), 7.95 (dd, 1H), 1.51 (s, 9H, 3CH₃); IR (KBr): 3056(s), 1797(vs, COO), 1735(vs, CONO), 1566(s), 1479(m), 1215(s), 1181(m), 1056(sh, s), 1016(s), 974(m), 831(s) cm⁻¹.

Anal. Calcd for $C_{11}H_{12}N_4O_3$: C, 53.21; H, 4.87; N, 22.57.

Found: C, 53.08; B, 4.74; N, 22.51.

EXAMPLE 8

[00169]   **Me₃C-COODhad.**
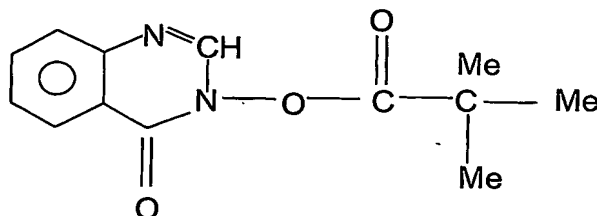
[00170]   Using the procedure described in Example 8, HODhad, (0.3708 g, 2.3 mmol), prepared as described in Example 5, was treated with pivaloyl chloride (0.31 ml, 2.53 mmol) in 15 ml of dry CH_2Cl_2 in the presence of TEA (0.48 ml, 3.45 mmol) to give 0.37 g (65%) of Me₃C-COODhad as a white solid. The analytical sample (0.26 g) was obtained after two recrystallizations from EtOAc-hexane as colorless crystals: mp 125-126.5°C; ^{1}H NMR(CDCl₃): δ8.91 (dd, 1H), 8.13 (dd, 1H), 8.07 (s, 1H, CH), 7.73 (dd, 1H), 1.48 (s, 911; 3CH₃); IR (KBr): 3036(w), 1794(s, COO), 1715 (vs, CONO), 1583(sh, m), 1464(m), 1427(m), 1296(s), 1059(vs), 1018(s), 824 (m) cm⁻¹.

Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.29; N, 17.00.

Found: C, 58.34; B, 5.28; N, 17.02.

EXAMPLE 9

O-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-triazin-3-yl)-1,1,3,3-
tetramethyluronium
Hexafluorophosphate (HDATU).

[00171]   Under an atmosphere of dry N_2, 0.22 ml (1 65 mmol) of TEA was
added to a suspension of HODhat (0.246 g, 1.5 mmol), prepared as in Example 4,
in 10 ml of dry CH_2Cl_2. After stirring for 5 min, the resulting light yellow clear
solution was cooled to 0°C in an ice-bath and 0.4209 g (1.5 mmol) of 1,1,3,3-
tetramethyluronium Hexafluorophosphate (TCFH) was introduced portion wise
with stirring. The stirring was continued for 30 minutes in an ice-bath and then at
room temperature for 1.5 hours. The precipitate which had separated was
collected by filtration and washed twice with methylene chloride and
recrystallized twice from MeCN-ether to give 0.42 g (69%) of analytically pure
hexafluorophosphate product as a white solid: mp 152°C (explodes); [1]H
NMR(CD$_3$CN): δ 9.19 (dd, 1H), 8.69 (dd, 1H), 8.13 (dd, 1H), 3.21 (s, 12H,
4CH$_3$); IR (KBr): 1738(vs), 1702(vs, CON), 1526(m), 1457(m), 1412(s), 1266(m),
1165(sh, s), 1072(m), 964(s), 850(sh, vs), 708(s) cm^{-1}.

Anal. Calcd for $C_{11}H_{15}N_6O_2PF_6$: C, 32.36; H, 3.70; N, 20.58.

Found: C, 32.14; H, 3.79; N, 20.47.

EXAMPLE 10

O-(3,4-Dihydro-4-oxo-5-azabenzo-1 ,3-diazin-3-yl)-1,1,3,3-tetramethyluronium Hexafluorophosphate (HDADU)

[00172]   Using the procedure of Example 10, the HODhad (0.2447 g, 1.5 mmol), prepared in Example 5, was treated with TCFH (0.4209 g, 1.5 mmol) in 10 ml of dry CH_2Cl_2 in the presence of TEA (0 30 ml, 2.1 mmol) to give 0.55 g (90%) of the above-identified hexafluorophosphate product as a white solid, which was recrystallized twice from MeCN-ether to give 0.48 g (79%) of analytically pure salt as a white solid: mp 203-205°C (dec.); ^{1}H NMR (CD_3CN): δ 8.91 (dd, 1H), 8.64 (s, 1H, CH), 8.22 (dd, 1H), 7.88 (dd, 1H), 3.17 (s, 12H, 4CH$_3$); IR (KBr): 1701(vs, CON), 1605(sh, m), 1528(s), 1471(s), 1416(sh, s), 1266(s), 1171(m), 1068(w), 965(s), 852(sh, vs), 718(s) cm^{-1}.

Anal. Calcd for $C_{12}H_{16}N_5O_2PF_6$: C, 35.38; 11, 3.96; N, 17.19.

Found: C, 35.51; H, 3.86; N, 17.33.

EXAMPLE 11

O-(3,4-Dihydro-4-oxo-5-azabenzo-1,2,3-trazin-3-yl)-1,1,3,3-bis(tetramethylene)- uronium Hexafluorophosphate (HDAPyU).

[00173]   To a mixture of 0.4923 g (3 mmol) of HODhat as prepared in Example 4 and 0.46 ml (3.3 mmol) of TEA in 25 ml of dry CH_2Cl_2 at 0°C, 1.0 g (3 mmol) of PyClu was added portion wise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 hour in an ice-bath and then at room temperature overnight. The clear light yellow mixture was diluted with CH_2Cl_2 to 50 ml and washed with ice cold water (2 x 15 ml) and dried over $MgSO_4$. The solvent was removed, and the oily residue was dissolved in 5 ml of MeCN to which 30 ml of ether was added, and the whole was stored at -20°C for several days until the oil solidified. The solid was collected by filtration and redissolved in 20 ml of CH_2Cl_2, and the solution washed with ice cold water (2 x 5 ml) and dried over $MgSO_4$. Removal of solvent gave a pink-yellow solid which was recrystallized from MeCN-ether to give 0.42 g (3 0%) of the above-identified uronium salt product as white crystals: mp 136.5°C (explodes); ^{1}H NMR (CD_3CN) δ9.17 (dd, 1H), 8.67 (dd, 1H), 8.11 (dd, 1H), 3.75 (t, 8H, 4NCH2), 1.97 (m, 8H, 4CH2); IR(KBr): 2985(m), 1734(vs, CON), 1679(vs), 1448(sh, s), 1341(m), 1169(m), 1072(m), 964(m), 846(sh, vs) cm^{-1}.

Anal. Calcd for $C_{15}H_{19}N_6O_2PF_6$: C, 39.13; H, 4.16; N, 18.26.

Found: C, 38.94; B, 4.08; N, 18.30.

EXAMPLE 12

[(3,4-Dihydro-4-oxo-5-azabenzo- 1,2,3-triazin-3-yl)oxy] tris(pyrrolidino)- phosphonium Hexafluorophosphate (PyDAOP)

[00174]　　　To a mixture of 0.2462 g (1.5 mmol) of HODhat, prepared as in Example 4 and 0.24 ml (1.65 mmol) of TEA in 10 ml of dry CH_2Cl_2 at 0°C, 0.6993g (1.5 mmol) of PyBrOP (tris(pyrrolidino) bromophosphonium hexafluorophosphate) was added portion wise with stirring under an atmosphere of dry N_2. Stirring was continued for 1 hour in an ice-bath and then at room temperature overnight. The clear light yellow mixture was diluted with CH_2Cl_2 to 25 ml and the solution washed with ice cold water (2 x 10 ml) and dried over $MgSO_4$. The resulting light yellow clear solution was treated with 50 ml of ether and the solid which separated was collected by filtration to give 0.45 g (54%) of analytically pure PyDAOP as a white solid after recrystallization from MeCN-ether: mp 149°C (dec.); ^{1}H NMR (CD_3CN): δ 9.20 (dd, 1H), 8.67 (dd, 1H), 8.13 (dd, 1H), 3.42 (td, 12H, 6NCH_2), 1.96 (td, 12H, 6CH_2); IR (KBr): 2982(m), 2893(m), 1742(vs, CON), 1566(m), 1462(sh, w), 1270(s), 1224(s), 1140(s), 1110(s), 1049(sh, m), 960(s), 839(sh, vs) cm^{-1}.

Anal. Calcd for $C_{18}H_{27}N_7O_2P_2F_6$: C, 39.34; H, 4.95; N, 17.84.

Found: C, 39.36; H, 5.09; N, 17.90.

EXAMPLE 13

[00175]

(Dmpp-OAt)
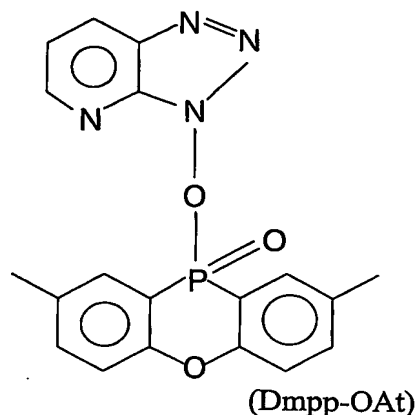
[00176]   **Method A.** To a suspension of 0.42 g (3.054 mmol) of HOAt in 20 mL of anhydrous CH_2Cl_2, there was added 0.43 mL (1 equiv.) of triethylamine with magnetic stirring. The resulting clear yellow solution was cooled in an ice bath under an atmosphere of N_2 and treated slowly with 0.85 g (1 equiv.) of 2,8-dimethylphenoxaphosphinic chloride.

[00177]   The reaction mixture was stirred at 0°C for 30 min, then at room temperature for 2 hours. After dilution with 30 mL of CH_2Cl_2, the organic phase was washed with H_2O, saturated aqueous NaCl (30 mL) and dried over $MgSO_4$. After removal of solvent with a rotary evaporator with the aid of a water aspirator, the residue was recrystallized from CH_2Cl_2-hexane to give 0.65 g (56.3%) of the above-identified phosphinic ester as white crystals, mp 164-166°C (dec); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.472 (s, 6), 7.218-7.559 (m, 5), 8.056-8.130 (d, 2), 8.319-8.368 (d, 2), 8.319-8.368 (d, 1), 8.698-8.729 (d, 1). IR (KBr): 1128-(P=O). Anal. Calcd for $C_{19}H_{15}N_4PO_3$: C, 60.32; H, 4.0; N, 14.81. HREIMS: M+ 378.0882; Found: 378.0877

[00178]   **Method B:** To a suspension of 1.25 g of HOAt in 20 mL of anhydrous CH_2Cl_2 there was added 0.623 g (1 equiv.) of imidazole with magnetic

stirring. The resulting white suspension was cooled in an ice bath under an atmosphere of N_2 and treated slowly with 2.56 g (1 equiv.) of 2,8-dimethylphenoxaphosphinic chloride. The reaction mixture was stirred at 0°C for 30 min, then at room temperature for 2 hours and diluted with 30 mL of CH_2Cl_2. The reaction mixture was filtered in a sintered glass funnel over anhydrous $MgSO_4$ under an atmosphere of N_2. After removal of solvent with a rotary evaporator with the aid of a water aspirator, the residue was recrystallized from CH_2Cl_2-hexane to give 2.86 g (82.3%) of the above-identified phosphinic ester as white crystals, for which the mp and NMR data agreed with the data reported above.

EXAMPLE 14

[00179]   Using the procedure described hereinabove, the following are also prepared:

[00180]   1.

(white solid)
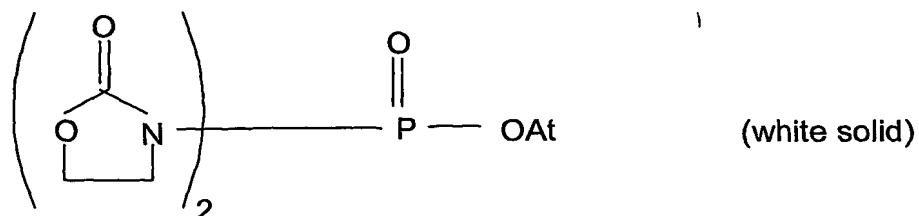
[00181]   2. OAt derivatives of
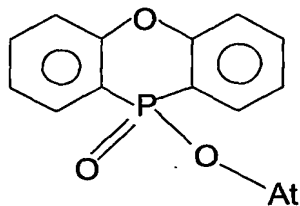
wherein the phenoxophosphinic acid products have the following substituents therein.

(a) 2,8-dibromo
(b) 2,8-dichloro
(c) 2,8-difluoro
(d) 2,8-dimethoxy
(e) 2,3,7,8-tetramethyl

EXAMPLE 15

Dmpp-4-Me-OAt

[00182]
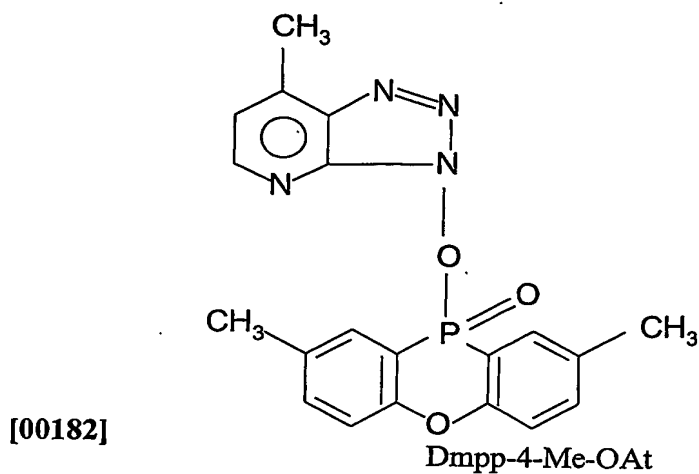
[00183]   Using method B of Example 14, and using 4-Me-HOAt instead of HOAt, the above-identified compound was made as a white solid.

EXAMPLE 16

Di-o-tolyl-phosphinyloxy-7-azabenzotriazole (DtpOAt)

[00184]   **A. Di-o-toylphosphine oxide.** Magnesium turnings (13.96 g) were added to 100 ml of anhydrous ether in a 3-neck flask fitted with a condenser, magnetic stirrer and a dropping funnel kept under an atmosphere of nitrogen. o-Bromotoluene (100 g, 0.579 mol) in 100 ml of ether was slowly added to the mixture. During the addition, the Grignard reaction was initiated and became so vigorous that ice bath cooling was needed frequently. After the addition was complete (40 min), the reaction mixture was refluxed for 15 min and then cooled with an ice bath and treated slowly with 30.8 ml (0.232 mol) of diethyl phosphite in 40 ml of ether. The mixture was refluxed again for 15 min and cooled with an ice bath. Two hundered and fifty milliliters of 10% HCl and 200 ml of water were added slowly to the cooled mixture with magnetic stirring. Ether was evaporated and the insoluble phosphine oxide was collected by filtration and recrystallized from CH_2Cl_2-hexane (a few drops of methanol may be added to help dissolve the solid) to give 39.47 g (73.9%) of the phosphine oxide as a pale yellow solid: mp 94°C, lit. mp 93-94°C, yield 57%; ^{1}H-NMR (60 MHz, $CDCl_3$): δ 2.376 (s, 6), 4.234 (s, 1), 7.19-7.94 (m, 8); IR (KBr): 2369 (P-H), 1168 (P=O) cm^{-1}.

[00185]   **B. Di-o-toylphosphinic acid.** A suspension of 15.04 g of di-o-toylphosphine oxide in 80 ml of 5N aqueous NaOH was treated with 40 ml of 30% H_2O_2 all at once and the resulting mixture was heated on a steam-bath for 20 min. A clear solution resulted and was filtered while hot. The filtrate was cooled in an ice bath and acidified slowly with concentrated HCl, which caused the precipitation of a white solid which was recrystallized from MeOH-ether to give 13.4 g (83.3%) of the phosphinic acid: mp 174-176°C, lit.175-177°C, yield 58-74%; ^{1}H-NMR (60 MHz, TFA): δ 2.369 (s, 6), 7.245-8.124 (m, 8); IR (KBr): 1143 (P=O) cm^{-1}.

[00186]   C. Di-o-tolylphosphinic acid chloride (DtpCl). Di-o-

tolylphosphinic acid (13.5 g) was slowly added to 50 ml of thionyl chloride with

cooling in an ice bath. The mixture was refluxed under a CaCl₂ drying tube for 3

hours. After removal of excess thionyl chloride by a water aspirator in a hood,

the oily residue was fractionally distilled to give 13.55 g (93.4%) of the

phosphinic acid chloride as a colorless oil (bp 158-165°C/0.1 mmHg) which

solidified quickly, lit. bp 150-160°C, yield 80.5%; ¹H-NMR (60 MHz, CDCl₃): δ

2.449 (s, 6), 7.05-8.08 (m, 8): IR (KBr): 1220 (P=O) cm⁻¹.

EXAMPLE 17

Di-o-tolyl-phosphinyloxy-7-azabenzotriazole (DtpOAt)

[00187]   The above-identified compound was prepared according to the

following scheme which is a variation of the procedure of Example 16:
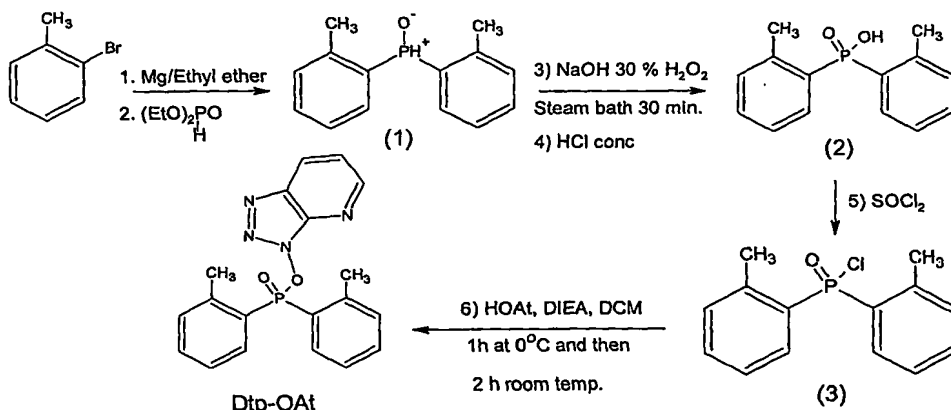
Scheme 1.

[00188]   A. Preparation of Di-o-tolylphosphine oxide (1) Magnesium

turnings (13.96 g) were added to 100 mL of anhydrous ether under a nitrogen

atmosphere in a 1 L 3-neck flask fitted with an efficient condenser, magnetic

stirrer and two dropping funnels. o-Bromotoluene (72 mL) was added to the

mixture. The rate of addition was adjusted to allow the ether to boil slowly. No

ice bath cooling was necessary during addition. After the addition was completed

the reaction mixture was refluxed for 2 hours and then cooled in an ice bath. Diethyl phosphite (30.8 mL) in 50 mL of ethyl ether was added from the second dropping funnel and the reaction mixture was stirred at room temperature overnight. Diethyl phosphite was distilled before use, bp 62-64 °C (5 mm Hg). Two hundered and fifty milliliters of 10% HCl and 200 mL of water were added slowly to the cooled mixture and the ether was removed with a rotavap. The insoluble material was isolated by filtration, dried and recrystallized from toluene to give 30.7 g (57.4%) of the phosphine oxide as pale yellow crystals, mp 95-97°C, lit. mp 94-95°C.

[00189]   **B. Preparation of di-*o*-tolylphosphinic acid (2)** - Di-*o*-tolylphosphine oxide (15 g) and 80 mL of 5 N NaOH were treated with 40 mL of 30% H_2O_2. The suspension was heated gently on a steam bath for 20 min. The heating was conducted carefully because the reaction could potentially be violent. Carrying out the reaction in a large beaker avoided loss of product if the reaction became violent. (The reaction mixture doesn't explode but a lot of foam and gas are developed.) The hot reaction mixture was added to an iced HCl solution (90 mL of conc. HCl plus 30 g of ice). The resulting white solid was isolated by extraction with chloroform (3x75 mL). The chloroform layer was washed with water and dried over magnesium sulfate. After the solvent was removed in vacuum the resulting solid was recrystallized from 95 % ethanol. The above amount of phosphine oxide was oxidized in two batches of 15 g each. The average yield of the two runs was 79 % for the pure phosphinic acid, mp 174-175°C, lit. mp 175-177°C.

[00190]   **C. Preparation of di-*o*-tolylphosphinic acid chloride (3).** Di-*o*-tolylphosphinic acid (22.5 g) was added in one portion to 75 mL of thionyl chloride and the reaction mixture heated under reflux for 3 hours. After removal of excess thionyl chloride, the residual oil was dissolved in dry dichloromethane (DCM), and the DCM removed at the rotavap, the operation being repeated three

times. The acid chloride which was obtained as an oil which solidified upon standing was used without further purification.

[00191]   **D. Preparation of Dtp-OAt-** To a suspension of 12.7 g of HOAt in 300 mL of anhydrous DCM was added 16.7 mL of DIEA. The resulting clear yellow solution was cooled in an ice bath under an atmosphere of nitrogen and treated with 25.8 g of di-*o*-tolylphosphinic acid chloride dissolved in 300 mL of DCM. The reaction mixture was stirred at 0°C for 1 hour and then at room temperature for 2 hours. The reaction mixture was diluted with 600 mL of DCM and washed with water and saturated aqueous NaCl and dried over MgSO$_4$. After removal of solvent with a rotary evaporator with the aid of a water aspirator, the residue was recrystallized from CH$_2$Cl$_2$/ethyl acetate to give 30.1 g (88 %) of the active ester as white crystals, mp 175-177°C, lit. mp 170-172°C.

[00192]   It was noted that this material has very low solubility in DMF. Some automatic peptide synthesizes, for example, the AIB 433 A peptide synthesizer, requires a solution of the activator in DMF to be placed in one of the reagent bottles which then delivers the activated species to the reaction vessel. The concentration of the reagent in solution has to be in the range of 0.3-0.8 M to guarantee the right concentration of the active species. Since the solubility of this reagent is so low in DMF, the yield for coupling two amino acids to form a peptide was low if this automatic synthesizer was used. However, a slight modification of the structure of the product of this example to make it soluble in DMF enhances its ability to couple amino acids to form peptides. The following example illustrates a structural modification of Dtp-OAt which dramatically enhances its solubility in DMF and other organic solvents.

EXAMPLE 18

5-*t*-Bu-Dtp-Oat

[00193]   The new coupling reagent was synthesized according to the
strategy outlined hereinbelow. Starting from the commercially available 4-*t*-Bu-
toluene the synthesis is completed in 5 steps.
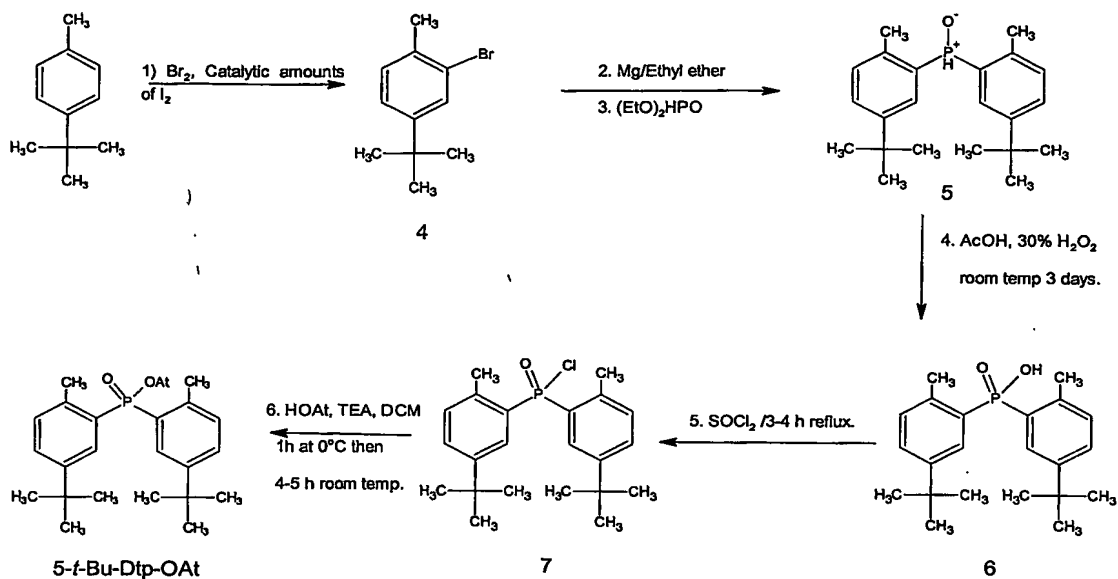
Scheme 2

[00194]   **A. Preparation of 2-bromo-4-*t*-butyltoluene (4).** The
bromination was carried out according to the procedure of Reich, et al. J. Med.
Chem. 1996, 39, 2781, the contents of which are incorporated by reference. To a
solution of 4-*t*-butyltoluene (90 mL, 0.518 mol) and two crystals of I_2 was added
dropwise bromine (27 mL, 0.524 mol). The reaction mixture was stirred at room
temperature for 3 hours and then poured into 1L of cold water. The mixture was
transferred to a separatory funnel and the lower layer was collected. The crude 2-
bromo-4-*t*-butyltoluene was washed with saturated $NaHCO_3$, brine and dried over
$MgSO_4$. The crude product was distilled under reduced pressure, first using a
water aspirator and then with a high vacuum oil pump. A first fraction was
collected up to b.p. 100^0C with the aid of the water aspirator and the residue was

distilled in high vacuum to give 90.5 g (77%) of the bromide as a colorless liquid, bp 61-65⁰C (3-5mm/Hg).

[00195]   **B. Preparation of bis-(5-*t*-butyl-2-methylphenyl)phosphine oxide (5).** This compound was obtained according to the procedure described for compound 1, described in Example 17. The phosphine oxide (5) was recrystallized from DCM/Hexane to give 15.8 g (32%) of the oxide as white crystals, mp 178-180⁰C; ^{1}H-NMR (400 MHz, CDCl$_3$) δ 1.317 (18 H, s), 2.334 (6 H, s), 7.715 (2 H, dd), 7.480 (2H, dt), 7.779 (2H, dd), 8.215 (1H, d, J$_{P-H}$ 474 Hz). IR (KBr) 2336(PH), 1187 and 1168 cm^{-1} (P=O) Anal. Calcd for C$_{22}$H$_{31}$OP: C, 77.16; H, 9.12. Found: C, 77.21; H, 8.98.

[00196]   **C. Preparation of bis-(5-*t*-butyl-2-methylphenyl)phosphinic acid (6).** To a solution of bis-(5-*t*-butyl-2-methylphenyl)phosphine oxide (6 g, 17.5 mmol) in 60 mL of acetic acid was added slowly 15 mL of 30 % H$_2$O$_2$. The reaction mixture was stirred at room temperature for three days and then poured into 300 mL of cold water. The white precipitate was isolated by filtration and recrystallized from ethanol/water to give 6 g (95%) of pure acid (6) as white crystals, mp 182-183⁰C; ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ 1.230 (18H, s), 2.172 (6H, s), 7.034-7.902 (6H). IR (KBr) 1162 cm^{-1}(P=O) Anal. Calcd for C$_{22}$H$_{31}$O$_2$P: C, 73.72; H, 8.72. Found: C, 74.00; H, 9.01.

[00197]   **D. Preparation of bis-(5-*t*-butyl-2-methylphenyl)phosphinic chloride (7).** The preparation was carried out as described for compound (3) as described in Example 17. The acid chloride was used in the next step without purification. ^{1}H-NMR (400 MHz, CDCl$_3$) δ 1.276 (18 H, s), 2.384 (6 H, s), 7.173-7.213 (2H, m), 7.493 (2H dt), 7.903 (2H, dd), IR (KBr) 1231 cm^{-1} (P=O).

[00198]   **E. Preparation of bis-(5-*t*-butyl-2-methylphenyl)phosphinyloxy-7-azabenzotriazole.** To a suspension of 0.76 g

(5.58mmol) of HOAt in 30 mL of anhydrous DCM was added 0.78 mL

(5.6mmol) of TEA. The resulting clear yellow solution was cooled in an ice bath

under an atmosphere of nitrogen and treated with 2 g (5.27mmol) of bis-(5-*t*-

butyl-2-methylphenyl)-phosphinic acid chloride dissolved in 20 mL of DCM.

The reaction mixture was stirred at 0°C for 1 hour and then at room temperature

for 4-5 hours. The reaction mixture was diluted with 40 mL of DCM and washed

with water and saturated aqueous NaCl and dried over $MgSO_4$. The solvent was

removed with a rotary evaporator with the aid of a water aspirator. The above

product was obtained as a white solid [2.3 g (86 %)] after recrystalization from

hexane and then from hexane containing a small amount of ethyl acetate: mp 86-

87°C, ^{1}H-NMR (400 MHz, $CDCL_3$) δ 1.271 (18 H, s), 2.687 (6 H, s), 7.264-7.298

(2H, m), 7.369 (1H, dd), 7.545 (2H dt), 7.974 (2H, dd), 8.309 (1H, dd), 8.729

(1H, dd). IR (KBr) 1242 cm^{-1} (P=O) HRFAB MS for $C_{27}H_{34}N_4O_2P$, M^+

477.2419, found 477.2400.

[00199]   Small-scale solubility tests showed that this compound (*t*-Bu-Dtp-

OAt) was highly soluble in DMF and thus could be used in the automated peptide

synthesizer.

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[00200]   As indicated hereinabove, without wishing to be bound, it is believed that during peptide coupling using uronium or phosphonium salts, the N-protected carboxylic acid first reacts with the coupling reagent to form an active ester, which then reacts with the amino component to give the corresponding amide. Therefore, the speed of formation of such an active ester is one of the important factors in evaluating the efficiency of the coupling reagent. The model chosen hereinbelow involved conversion of N-benzyloxycarbonyl-α-aminoisobutyric acid (CBZ-Aib-OH) to the corresponding active ester in both DMF and CDCl$_3$, as described in the chemical equation hereinbelow.

$$\text{CBZ} \text{---} \text{Aib} \text{---} \text{OH} \xrightarrow[\text{DIEA}]{\text{coupling reagent}} \text{CBZ} \text{---} \text{Aib} \text{---} \text{Oxt}$$

xt = At
xt = Bt
xt = Dhbt
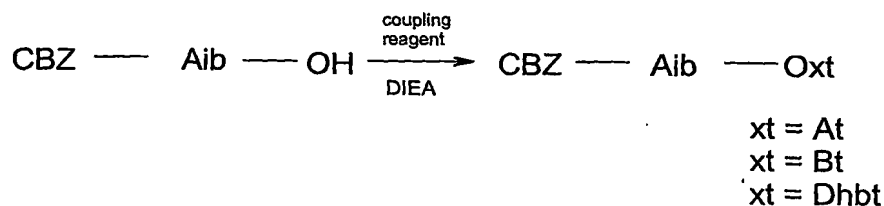
[00201]

[00202]   In the study, a comparison was made with derivatives of HOBt, HOAt with HODhbt:

HOBt   and   HOAt   and

HODhbt
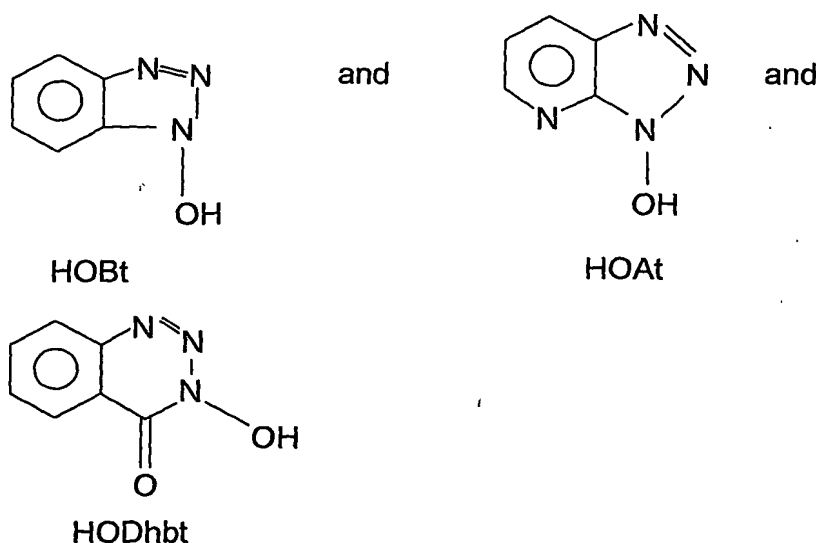
[00203]   The benzylic CH_2 units of CBZ-Aib-OH ($\delta 5.09$) and active ester ($\delta 5.20$) were monitored by NMR. Assignment of the peak at $\delta 5.20$ in the active esters was confirmed by authentic synthesis.

[00204]   Because of the sterically hindered carboxyl group of Aib, activation in the above equation is slow relative to the case of the proteinogenic amino acids allowing different coupling reagents to be more closely differentiated.

[00205]   The procedure is described in Example 19 hereinbelow.

EXAMPLE 19

[00206]   (a)   Di-o-tolylphosphinyloxybenzotriazole (DtpOBt). The preparation was carried out similarly to that as described for preparing the product of Example 16: HOBt (0.125 1g, 1mmol) was treated with Dtp-Cl (0.267 g, 1 mmol) in the presence of DIEA (0.21 ml, 1.2 mmol) for 5 hours in 10 ml of dry CH_2Cl_2 to give 0.24 g (66%) of white solid after work-up. Recrystallization from CH_2Cl_2 -hexane gave 0.18 g of an analytically pure sample of the ester as colorless crystals: mp 198-200°C; ^{1}H-NMR (CDCl$_3$): δ8.06 (m, 3), 7.30 (m, 6), 2.62 (s,6); IR (KBr): 3065 (W), 1593 (s), 1457 (s), 1362 (m), 1278 (m), 1230 (vs), 1151 (s), 1084 (sh, s), 812 (vs), 774 (sh, vs), 704 (m) cm^{-1}. Anal. Calcd for $C_{20}H_{18}N_3O_2P$: C 66.10; H, 4.92; N, 11.56. Found C, 65.75; H, 4.97; N, 11.41.

[00207]   (b)   3-[di-o-tolyl)phosphinyloxy]-3,4-dihydro-4-oxo-2,3 benzotriazine] (DtpODhbt) was made according to method A described for DmppOAt (Method A) in Example 14 using HODhbt, which was prepared similarly to the method described in Ex. 4 using HOBt instead of HOAt and using di-o-tolylphosphinic chloride. The product was obtained as white crystals (76.5%) after recrystallization from ethyl acetate-ether: mp 178-179°C(dec); ^{1}H-NMR (200 MHz, CDCl$_3$): δ2.58 (s,6), 7.28 (m,4), 7.487-7.570 (m,2), 7.75 (m,1), 7.90-8.15 (m,4), 8.351 (dd,1); IR (KBr): 1709 (C=O), 1240 (P=O) cm^{-1} . Anal. Calcd for $C_{21}H_{18}N_3PO_3$; C, 64.45; H, 4.64; N, 10.74. Found: C, 64.49; H. 4.54; N, 10.69.

[00208]   (c)   Active Ester Formation. To a solution of 0.1 mmol of CBZ-Aib-OH and 0.1 mmol of the appropriate coupling reagent in 0.5 ml of CDCl$_3$ or DMF, was added 0.1 mmol of DIEA. The mixture was immediately transferred to an NMR tube which was placed in the probe of a Hitachi R-1200 (60 MHz) NMR instrument. Integration of the ^{1}H-NMR peaks at δ5.1 (acid) and 5.2 (active ester) as the reaction progressed at the probe temperature (37°C)

allowed for rough determination of the relative rates. The results are given in Table 1 and represent the averages of at least two runs.

[00209]

Coupling Reagent	$t_{1/2}$ (DMF), min	$t_{1/2}$ (CDCl$_3$), min
DEPOAt	<2	2-3
DPOPOAt	<2	2-3
DEPDBt	7-8	45-47
DPOPDBt	<2	<2
DtpOBt	65-70	11-12h
N-HATU	<2	14-15
N-HAPyU	<2	<2
HDTU	<2	<2
N-HBTU	<2	>24h

[00210]   In the examples that follow, it is to be understood that the amino acid sequence is presented in the amino to carboxy direction, from left to right.

EXAMPLE 20

[00211]   To test for configuration control, three different peptides, CBZ-
Phe-Val-Pro-NH$_2$, CBZ-Gly-Phe-Pro-NH$_2$, and CBZ-Gly-Gly-Val-Ala-Gly-Gly-
OMe (SEQ ID NO:1) were prepared and the loss of configuration during the
coupling was determined. An exemplary procedure is given for a dipeptide as
follows:

[00212]   A. CBZ-Phg-Pro-NH$_2$ As a standard protocol, 35.6 mg (0.125
mmol) of CBZ-Phg-OH, 14.3 mg (0.125 mmol) of H-Pro-NH$_2$, and 0.25 mmol of
base in 1 ml of DMF or other solvent was treated with 0.125 mmol of an
appropriate coupling reagent at 0°C. The mixture was stirred at 0^{0}C for 1 hour and
at room temperature overnight. The resulting mixture was diluted with 25 ml of
EtOAc and washed with HCl (2 x 10 ml), 10% NaHCO$_3$ solution (2 x 10 ml) and
brine (2 x 10 ml), and dried over MgSO$_4$. An oily peptide residue was obtained
after removing solvent. The oily residue was redissolved in 1 ml of CH$_2$Cl$_2$ and
20 ml of hexane was added. A white solid was obtained after filtration. About 5
mg of this crude product, usually containing both LL- and DL- forms of CBZ-
Phg-Pro-NH$_2$, was dissolved in 4 ml of MeCN and directly analyzed by HPLC as
described in Wenschuh, et al., J. Org. Chem., 1995, 62, 405, the contents of which
are incorporated by reference.

[00213]   B. CBZ-Phe-Val-Pro-NH$_2$. The standard protocol as described
above for CBZ-Phg-Pro-NH$_2$ was followed.

[00214]   C. CBZ-Gly-Phe-Pro-NH$_2$. The standard protocol as described
above for CBZ-Phg-Pro-NH$_2$ was followed.

[00215]   D.CBZ-Gly-Gly-Val-Ala-Gly-Gly-OMe (SEQ ID NO 1.) As
described for CBZ-Phg-Pro-NH$_2$, a solution of 45.6 mg (0.125 mmol) of CBZ-

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Gly-Gly-Val-OH, 31.7mg (0.125 mmol) of H-Ala-Gly-Gly-OMe•HCI and 49.6µl
(0.375 mmol) of TMP in 1 ml of DMF was treated with 0.125 mmol of an
appropriate coupling reagent at 0°C. The resulting mixture was stirred at 0°C for 1
hour and then at room temperature overnight. The crude hexapeptide methyl ester
was isolated by evaporation of solvent followed by direct column chromatography
using MeOH/CHCl₃/HOAc (3:7:0.1) as eluate. The crude material from the
column, containing both LL- and DL-forms of hexapeptide, was examined by
HPLC as described in Carpino, J. Org. Chem., 1994, 59, 695, the contents of
which are incorporated by reference.

[00216]   The results are given in Table 2

Table 2. Effect of Coupling Reagent on Loss of Configuration During [2+1]
Coupling Leading to CBZ-Phe-Val-Pro-NH₂, CBZ-Gly-Phe-Pro-NH₂ and [3+3]
Coupling Leading to CBZ-Gly-Gly-Val-Ala-Gly-Gly-OMe in DMF with 2 eqs.
TMP as base.

Coupling Reagent	CBZ-Phe-Val-Pro-NH₂	CBZ-Gly-Phe-Pro-NH₂	CBZ-Gly-Gly-Val-Ala-Gly-Gly-OMe
DEPOAt	0.9 (0.9)	<0.1	<0.1
DmppOAt	3.6 (2.0)	0.3	
DtpOAt	2.9 (1.4)	0.4	
N-HATU	5.0 (1.8)	1.1 (0.9)	2.4
DEPDBt	3.5	0.3	2.4
DtpODhbt	4.3 (3.6)		
HDTU	8.5 (4.0)		3.3
DtpOBt	11.4	2.2	
N-HBTU	14.2	3.6	8.2

ᵃFigures in parenthesis refer to identical runs but with 1 eq. of the appropriate
HOXt e.g., HOAt, HOBt or HODhbt added.

[00217]   As confirmed by the data herein, the new phosphorous-based OAt
derivatives of the present invention are much more effective in preserving
configuration than any of the other tested reagents, including N-HATU. The best
of the previously-described uronium/guanidinium salts (N-HAPyU) sometimes

equals the results of the new phosphorus esters, but where differences are observed, the latter have proved more effective in every case examined to date.

[00218]   Among the results obtained from these data involving N-HAPyU, it was found that for the new reagents DEPOAt and DPOPOAt, a one-equivalent excess of proline serving as base gave the lowest epimerization levels yet observed for the tripeptide CBZ-Phe-Val-Pro-NH$_2$ in DMF (0.5% LDL-isomer). Upon switching to other solvents, even greater differences were found between the new phosphorus reagents of the present invention and the related uronium/guanidinium salts. For example, in the special structure-breaking combination solvent trifluoroethanol/trichloromethane (TFE, TCM, 1:3), 12.2% of the LDL-form was obtained for DPOPOAt/TMP as opposed to 38.5% for N-HATU/TMP. In CH$_2$Cl$_2$ in the presence of TMP, 2.2% (DEPOAt) and 2.9% (DPOPOAt) were clearly better than values observed for guanidinium reagents N-HATU (9.3%) and N-HAPyU (5.3%).

[00219]   In order to determine the coupling efficiency of diphenyl phosphorochloridate (DPOPCl) various coupling conditions were used. It was noted that without additive, DPOPCl gave only a very small amount of the desired peptide for both diisopropylethylamine and trimethylpyridine. If one equivalent of HOAt (1-hydroxy-7-azabenzotriazole) was present, the results were acceptable. Indeed the mixture DPOPCl/HOAt/Base, which contains DPOPOAt as the active species, gave results which are comparable to those obtained with the isolated reagent DPOPOAt.

[00220]   The Tripeptide CBZ-Phe-Val-Pro-NH$_2$ was also chosen as a model to study loss of configuration associated with use of various reagents of the present invention under solid phase conditions. In comparison with results obtained in solution, the data show how much more difficult it is to maintain configuration in the solid phase mode. The system involved overnight coupling

of four equivalents of CBZ-Phe-Val-OH onto H-Pro-PAL-PEG-PS in the presence of 8 eqs. of trimethylpyridine in DMF, cleavage of the tripeptide from the resin via trifluoroacetic acid/H$_2$0 (9:1) over a period of 1 hour and separation of the diastereomers as described for the solution system. Although extensive loss of configuration occurs in all cases, the data show that the effectiveness of the various coupling reagents follows the same order as in solution, thus coupling reagent/LDL (%): DEPOAt/11.6, N-HAPyU/13.0, N-HATU/13.6, DPOPODBt/ 19.4, DEPDBt/19.5, HDTU/24.2, N-HBTU/29.8.

EXAMPLE 21

ACP Assembly via Stepwise Coupling on Solid Phase

[00221]   In order to demonstrate the suitability of the organophosphorus-
based coupling reagents and compare their performance with that of the
corresponding uronium/guanidinium analogs in solid phase syntheses, several
syntheses of the ACP decapeptide segment 65-74 (H-Val-Gln-Ala-Ala-Asp-Tyr-
Ile-Asn-Gly-NH$_2$) (SEQ. ID. NO. 2) were carried out.

[00222]   The protocol is as follows: 150 mg of Fmoc-Gly-PAL-PEG-PS
resin (0.19 mmol/g, 0.0285 mmol) in a 10-ml disposable syringe fitted with a
Teflon filter was washed with CH$_2$Cl$_2$ (3 x 10 ml) and DMF (3 x 10 ml) and
deprotected with 20% piperidine in DMF (10 ml) for 7 min. The deprotected resin
was washed with DMF (3 x 10 ml), CH$_2$Cl$_2$ (3 x 10 ml) and again DMF (3 x 10
ml). Preactivation was carried out for 7 min using 25.5 mg (0.04 mmol, 1.5 equiv)
of Fmoc-Asn(Trt)-OH, 15.75 mg (0.04 mmol, 1.5 equiv) of DPOPOAt and 14.89
µl (0.09 mmol, 3 equiv) of DIEA (diisopropylethylamine) in 0.15 ml of DMF in a
4-ml vial. Following the requisite preactivation period (7 min), the solution of the
activated amino acid was added to the resin. The small vial was washed with 0.04
ml of DMF, and the washing was also added to the above resin. The resulting
resin mixture was allowed to react at room temperature for 1.5 min.. The loaded
resin was washed with DMF (3 x 10 ml) and the Fmoc group was deblocked with
10 ml of 20% of piperidine in DMF for 7 min. Washing the deblocked resin with
DMF (3 x 10 ml), CH$_2$Cl$_2$ (3 x 10 ml) and DMF (3 x 10 ml) was followed by an
analogous coupling step with Fmoc-Ile-OH. Other amino acids were coupled
similarly and after the last coupling with Fmoc-Val-OH and deblocking of the
Fmoc group with 20% piperidine in DMF, the loaded resin was washed with
DMF (3 x 10 ml), CH$_2$Cl$_2$ (3 x 10 ml), EtOH (5 ml) and ether (5 ml). The resin
was then treated with 10 ml of 90% aqueous trifluoroacetic acid for 2 hours,
filtered, and washed on the filter with 10 ml of 10% trifluoroacetic acid in CH$_2$Cl$_2$
and 10 ml of CH$_2$Cl$_2$. The combined filtrates were evaporated to dryness. The

crude product was washed four times with anhydrous ether and separated by centrifugation. The yield was calculated by the weight of the crude product. For analysis 1 mg of the crude product was dissolved in 1 ml of 0.1% aqueous trifluoroacetic acid and injected directly onto the HPLC column for analysis. The procedure was repeated using the same coupling agent until the peptide of SEQ ID NO: 2 was prepared.

[00223]   This procedure was repeated using each of the coupling agents listed in Table 3 for the preparation of the peptide of SEQ. ID. NO. 2. The results are given in Table 3.

[00224]   **Table 3** Distribution of Products, Including Various Deletion Peptides, According to HPLC Analysis for the Assembly of ACP (65-74) via HOAt- and HODhbt-Based Coupling Reagents

Entry	Coupling Method	Equiv. of Reagents	Preactivation Time (mm)	Coupling (min)	ACP (%)	-2Ile (%)	-Ile72 (%)	-Ile69 (%)	-Val (%)	-Ala (%)	-Asn (%)
1	DEPOAt	1.5	7	1.5	84	-	2	2	1	4	5
2	DPOPOAt	1.5	7	1.5	85	-	2	1	2	6	3
3	DEPDBt	1.5	7	1.5	6	9	13	19	3	-	1
4	DPOPOBt	1.5	7	1.5	23	21	26	19	1	1	2
5	N-HATU	1.5	7	1.5	85	-	1	1	3	-	10
6	HDTU	1.5	7	1.5	38	15	15	26	-	-	5
7	DEPOAt	1.5	0	1.5	86	-	4	2	2	3	1
8	DPOPOAt	1.5	0	1.5	81	-	4	1	1	7	-
9	DEPDBt	1.5	0	1.5	<1c	-	-	-	-	-	-
10	DPOPOBt	1.5	0	1.5	29	17	25	17	2	-	3
11	N-HATU	1.5	0	1.5	87	-	3	1	2	-	6
12	HDTU	1.5	0	1.5	30	15	19	22	3	-	4

*A reversed-phase C-18 column was used with elution by a linear gradient over 20 mm of 0.1% TFA in MeCN and 0.1% TFA from 1:19 to 1:1, flow rate 1.0 ml/mm. ᵇCouplings were carried out in DMF in the presence of 2 equivalents of di-isopropylethylamine per equivalent of Fmoc-amino acid/coupling reagent. ᶜOnly a trace of the desired product was obtained.

[00225]   In this experiment, coupling times are shortened and excesses of reagents are reduced in order to bring out differences among the various reagents studied. Under these conditions, incomplete incorporations were detected for Asn onto Gly, Ile onto Asn, Ile onto Asp, Val onto Gln and Ala onto Ala or Asp. Analysis of the chromatograms indicated that the new HOAt-based organophosphorus reagents are as effective as N-HATU under these so-called "1.5 x 1.5" conditions with or without preactivation. Under normal coupling conditions such as 4 eqs excess amino acid/30 min. coupling time, all reagents worked well with the exception of HDTU.

EXAMPLE 22

[00226]   In the following example, reactions of the hindered active esters CBZ-Aib-OXt with p-chloroaniline (PCA) were studied in CDCl₃. Approximate halftimes were determined by proton NMR analysis according to the disappearance of the benzylic CH₂ unit (δ 5.2) of the active esters and appearance of the benzylic CH₂ residue (δ 5.5) of the product.

[00227]   CBZ-Aib-OXt Esters. The reaction of Z-Aib-ODhat with PCA is taken as an example to demonstrate the standard method used in order to follow aminolysis via an NMR protocol: To a solution of 47.9 mg (0.125 mmol) of CBZ-Aib-ODhat in 0.5 ml of CDCl₃, was added 15.6 mg (0.125 mmol) of p-chloroaniline (PCA). The mixture was immediately transferred to an NMR tube, which was placed in the probe of a Hitachi R-1200 (60 MHz) instrument. Integration of the ¹H NMR peaks at δ 1.8 (CH₃ residue of ester CBZ-Aib-ODhat) and 1.57 (CH₃ residue of amide CBZ-Aib-PCA) [or peaks at δ5.2 (benzylic CH₂ unit of ester CBZ-Aib-ODhat) and 5.05 (benzylic CH₂ unit of the product amide)] as the reaction progressed at the NMR probe temperature (37°C) allowed for rough determination of the relative rates. The results given in Table 4 are the average of at least two runs.
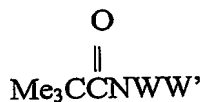
[00228]   Table 4 Approximate Halftimes for Disappearance of CBZ-Aib-OXt in CDCl₃ in the Presence of p-Chloroaniline

CBZ-Aib-OXt	$t_{1/2}$ (min)
CBZ-Aib-ODhat	8-9
CBZ-Aib-OAt	9-10
CBZ-Aib-ODhbt	12-13
CBZ-Aib-ODhad	70
CBZ-Aib-OBt	210

[00229]   It was found that the ODhat ester is slightly more reactive even
than the OAt ester, which was previously found to be the most reactive derivative
among these esters. Interestingly, despite the structural similarity between
HODhat, i.e., 3-hydroxy-4-oxo-3,4-dihydro-5-azabenzo-1,2,3-triazine, and
HODhad, i.e., 3-hydroxy-4-oxo-3,4-dihydroxy 5-azabenzo-1,3-diazine the
reactivities of the corresponding active esters are very different. Without wishing
to be bound, it is believed this may be due to the presence or absence of additional
neighboring group effects promoted by the presence or absence of a nitrogen atom
substituted at the 2-position. On the other hand, comparison of the OBt and
ODhad esters demonstrates the importance of the neighboring carbonyl group.

EXAMPLE 23

[00230]   In this example, a second model was used. Pivaloyl esters in the presence of various comparative coupling agents were treated with benzylamine and *N*-methylbenzylamine, which led to the formation of amides of the following formula:

$$O$$
$$\|$$
$$Me_3CCNWW'$$

XXV(a):   W=H, W'=benzyl

XXV(b):   W=Me, W'=benzyl.

[00231]   Approximate halftimes for these reactions were determined by proton NMR analysis, according to the disappearance of the methyl peak (δ 1.5) for pivaloyl-OXt and the appearance of the methyl peak for products XXV_a(δ 1.2) or XXV_b(δ 1.3). The protocol is as follows:

[00232]   **$Me_3CCOOXt$ Esters.** As in the case with Example 22, the reaction of pivaloyl ester with N-methyl benzylamine is taken here as an example to demonstrate the methodology used: To a solution of 31.0 mg (0.125 mmol) of $Me_3CCOODhat$ in 0.5 ml of $CDCl_3$, was added 15.1 mg (16.1 µl, 0.125 mmol) of $PhCH_2NHMe$. The mixture was immediately transferred to an NMR tube, which was placed in the probe of a Hitachi R-1200 (60 MHz) instrument. Integration of the ^1H NMR peaks at δ 1.5 (CH_3 residue of ester $Me_3CCOODhat$) and 1.3 (CH_3 residue of amide) as the reaction progressed at the NMR probe temperature (~37°C) allowed for rough determination of the relative rates. The results given in Table 5 are the average of at least two runs.

[00233]   The results are tabulated in Table 5.

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[00234]   **Table 5** Approximate Halftimes for Disappearance of Me_3C-CO-Oxt in $CDCl_3$

$Me_3CCOOXt$	$t_{1/2}$ $(PhCH_2NH_2)$	$t_{1/2}$ $(PhCH_2NHMe))$
$Me_3CCOODhat$	<1 min	<2 min
$Me_3CCOOAt$	<1 min	7-8 min
$Me_3CCOODhbt$	<1 min	18-20 min
$Me_3CCOODhad$	<1 min	35-40 min
$Me_3CCOOBt$	<1 min	4.5 hr

[00235]   In the case of benzylamine all reactions were rapid whereas in the case of the more hindered N-methyl derivative, clear reactivity differences were seen according to the following order: ODhat>OAt>ODhbt>ODhad>OBt. Again the greater reactivity of the HODhat ester relative to that derived from HOAt is seen.

EXAMPLE 24

[00236]   Another comparative study was run to compare relative rates of coupling processes involving the reaction of CBZ-Aib-OH with p-chloroaniline (PCA) in the presence of a coupling reagent. Because formation of intermediate Z-Aib-OXt is usually very fast, halftimes are determined by disappearance of the benzylic CH_2 residue (δ 5.2) of the active ester and appearance of the benzylic CH_2 unit (δ 5.05) of product CBZ-Aib-PCA unless otherwise noted. The protocol for the preparation of this product is the same as described in Example 23. Approximate halftimes are collected in Table 6. In this case various solvent systems were examined.

[00237]   **Table 6** Approximate Halftimes for Disappearance of [CBZ-Aib-Oxt] in Various Solvent Systems in the Presence of p-Chloroaniline

Coupling Reagent	$t_{1/2}$ (CDCl$_3$)	$t_{1/2}$ (CD$_3$CN)	$t_{1/2}$ (DMF)	$t_{1/2}$ (DMF/CDCl$_3$)[a]
HDATU	<3 min	70-75 min		30-40 min
N-HATU	18-24 min	90-95 min	75-85 mm	40-45 min
HDTU	20-25 min	4.5-5 hr	6-7 hr	2.5-3 hr
N-HBTU	3.5-4hr		10-11 hr[b]	

[a]1:1 mixture of DMF/CDCl$_3$. [b]In this case, the halftime is determined by disappearance of acid Z-Aib-OH and appearance of both intermediate active ester and amide CBZ-Aib-PCA.

[00238]   Interestingly in all solvent systems examined except for DMF, the new coupling reagent was found to be more reactive than N-HATU. In CDCl$_3$, HDATU is at least six times as reactive as N-HATU and about eight times as reactive as HDTU. So far, in every case tested HDATU was shown to be significantly more reactive than HDTU.

EXAMPLE 25

[00239]   In order to test the configuration-retention effectiveness of the additives HODhat and HODhad, and the coupling reagents HDATU, HDADU, HDAPyU, HDPyU, PyDAOP, and PyDOP, the following model peptide systems were examined. These involve a [1+1] stepwise coupling, and three [2+1] and one [3+3] segment couplings.

CBZ-Phg-Pro-NH$_2$,   CBZ-Phe-Val-Pro-NH$_2$,   CBZ-Gly-Phe-Pro-NH$_2$

XXVI   XXVII   XXVIII

CBZ-Gly-Gly-Val-Ala-Gly-Gly-OMe (SEQ ID NO 1), and H-Gly-Leu-Phe-OBzl

XXIX   XXX

[00240]   Test couplings were carried out as described previously in Example 22 and in L.A. Carpino, et al., J. Org. Chem. 1990, 61,2463, for CBZ-Phg-ProNH$_2$, CBZ-Phe-Val-Pro-NH$_2$, CBZ-Gly-Phe-Pro-NH$_2$ and CBZ-Gly-Gly-Val-Ala-Gly-Gly-OMe substituting the coupling reagent listed hereinbelow in the tables and the protocols therein, the results of which are incorporated by reference. For Boc-Gly-Leu-Phe-OBzl, (Bzl = benzyl) 60.6 mg (0.21 mmol) of Boc-Gly-Leu-OH, 85.45 mg (0.20 mmol) of H-Phe-OBzl•pTsOH and 0.22 mmol of an appropriate coupling additive (HOXt) were dissolved in 1 ml of DMF or trifluroethanol/trichloromethane (1:3 v/v). To the mixture, a solution of 34.2 mg (0.22 mmol) of EDC (1-ethyl-3-3'-(dimethylamino)-propyl)carbodiimide in 1 ml of DMF or trifluroethanol/trichloromethane was added and the whole mixture was stirred at room temperature overnight. The resulting mixture was diluted with 25 ml of EtOAc and washed with 1 N HCl (2 x 10 ml), 10% NaHCO$_3$ (2 x 10 ml) and brine (2 x 10 ml), and dried over MgSO$_4$. After removal of solvent, the solid was weighed to determine the yield. The solid was then stirred with 2 ml of 50% trifluoroacetic acid in a methylene chloride solution for 2 hours to deblock the BOC-group. The trifluoroacetic acid and CH$_2$Cl$_2$ were then removed in vacuo

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and 20 ml of anhydrous ether was added to the oily residue, and the mixture was stored at room temperature overnight. The white precipitate which had separated was collected by filtration and washed with ether. About 5 mg of the crude product, containing both LL- and DL- forms of XXX was dissolved in 4 ml of MeCN and directly analyzed by HPLC using a reversed-phase Waters C-18 column, with elution by a linear gradient over 20 min of 0.1% trifluoroacetic acid in MeCN and 0.1% aqueous TFA from 1:9 to 11:9, at a flow rate of 1.0 ml/min. The retention times for the LL and DL-forms of XXX were 17.3 and 17.9 min, respectively. The results are as follows:

[00241]   For the sensitive coupling of the urethane- protected CBZ-Phg-OH to H-Pro-NH$_2$ to give XXVI, HDATU was more effective in preserving configuration than HDTU and N-HBTU, but not better than N-HATU. Curiously with this system, use of the base diisopropylethyl amine (DIEA) proved more satisfactory than collidine (TMP), a result that is rarely observed in the case of the corresponding segment couplings. Results are collected in Table 7.

[00242]　　　　　Table 7 Effect of Coupling Reagent, Base and Solvent on the
Preservation of Configuration during the Formation of XXVI via [1+1] Coupling

Coupling Reagent (%)	Additive	Base (Eq.)	Solvent	Yield (%)	DL
HDATU		DIEA(2)	DMF	83.9	4.8
HDTU		DIEA(2)	DMF	78.4	12.8
N-HATU		DIEA(2)	DMF	71.7	2.8
N-HBTU		DIEA(2)	DMF	81.3	6.3
HDATU		TMP(2)	DMF	87.5	6.0
HDTU		TMP(2)	DMF	80.7	16.0
N-HATU		TMP(2)	DMF	90.8	3.8
N-HBTU		TMP(2)	DMF	85.4	8.7
DCC	HODhat(1)	TMP(1)	TFE/TCM[a]	74.8	0.4
DCC	HODhbt(1)	TMP(1)	TFE/TCM[a]	71.8	0.8
DCC	HOAt(1)	TMP(1)	TFE/TCM[a]	69.2	0.3

[a]In this case, 1.3 ml of trifluoroethanol-chloroform (1:3 v/v) was used as solvent.

[00243]　　　　　With carbodiimide reagents in the solvent
trifluroethanol/trichloromethane, HODhat was even more effective than HODhbt.
Thus, EDC/HODhat gave 0.5% of the DL-isomer, whereas EDC/HODhbt led to
1.3% of the same form. For DCC/HODhat and DCC/HODhbt in the presence of
1 equivalent of trimethylpyridine, the figures were 0.4% and 0.8%, respectively.

[00244]　　　　　For the well-studied segment coupling of CBZ-Phe-Val-OH to H-
Pro-NH$_2$ leading to tripeptide XXVII, the results are tabulated in Table 8.

[00245]   **Table 8** Effect of Coupling Reagent, Base and Solvent on the

Preservation of Configuration during the Formation of XXVII via [2+1] Coupling

Coupling Reagent	Additive	Base (Eq.)	Solvent	Yield (%)	LDL (%)
HDATU		DIEA(2)	DMF	85.4	15.1
HDTU		DIEA(2)	DMF	81.0	13.3
HDADU		DIEA(2)	DMF	72.4	27.6
N-HATU				DIEA(2)	DMF
N-HBTU				DIEA(2)	DMF
HDATU		TMP(2)	DMF	88.8	8.7
HDTU		TMP(2)	DMF	86.4	8.5
HDADU		TMP(2)	DMF	83.8	18.6
N-HATU			TMP(2)	DMF	80.1
N-HBTU			TMP(2)	DMF	81.2
HDATU	HODhat(1)	TMP(2)	DMF	68.0	7.3
HDTU	HODhbt(1)	TMP(2)	DMF	65.0	4.0
N-HATU	HOAt(1)			TMP(2)	DMF

* DIEA= diisopropylethylamine; TMP=trimethylpyridine

[00246]   For the rather insensitive case of the segment coupling of Z-Gly-
Phe-OH to H-Pro-NH$_2$, the results paralleled those for XXVII. Results are
presented in Table 9.

[00247]   **Table 9.** Effect of Coupling Reagent, Base and Solvent on the
Preservation of Configuration during the Formation of XXVIII via [2+1]
Coupling[a]

Coupling Reagent	Additive	Base (Eq.)	Solvent	Yield (%)	LDL (%)
HDATU		DIEA(2)	DMF	96.5	0.5
HDTU		DIEA(2)	DMF	82.0	1.5
HDADU		DIEA(2)	DMF	92.8	7.2
N-HATU		DIEA(2)	DMF	86.0	0.8
N-HBTU		DIEA(2)	DMF	84.8	5.9
PyDAOP		DIEA(2)	DMF	89.9	0.8
PyDOP		DIEA(2)	DMF	94.3	0.7
HDATU		TMP(2)	DMF	94.1	1.0
HDTU		TMP(2)	DMF	98.3	1.7

HDADU			TMP(2)	DMF	86.6	8.4
N-HATU			TMP(2)	DMF	96.5	1.1
N-HBTU			TMP(2)	DMF	88.2	3.6
HDAPyU			TMP(2)	DMF	78.8	1.2
HDPyU			TMP(2)	DMF	80.9	1.4
PyDAOP			TMP(2)	DMF	84.3	1.8
PyDOP			TMP(2)	DMF	90.4	1.7
HDATU			DIEA/TMP[b]	DMF	85.8	0.8
HDTU			DIEA/TMP[b]	DMF	84.8	1.5
N-HATU			DIEA/TMP[b]	DMF	76.4	0.9
DIC(diisopropylcarbodimide)	HODhat(1)			DMF	86.2	0.5
DIC	HODhbt(1)			DMF	81.4	0.8
DIC	HODhad(1)			DMF	74.3	8.2
DIC	HOAt(1)			DMF	82.3	0.4
EDC HCl	HODhat(1)	TMP(1)		DMF	94.0	1.8
EDC HCl	HODhad(1)	TMP(1)		DMF	85.2	2.2
EDC HCl	HOAt(1)	TMP(1)		DMF	89.3	3.3
EDC HCl	HODhat(1)	TMP(2)		DMF	90.1	2.8
EDC HCl	HODhbt(1)	TMP(2)		DMF	86.1	1.6
EDC HCl	HOAt(1)	TMP(2)		DMF	91.0	1.7
EDC	HODhat(1)			DMF	88.8	3.4
EDC	HODhat(1)			TFE/TCM[c]	80.2	2.5

[a] Abbreviations as given hereinabove. [b] In this case, a combination of 1 equiv of DIEA and 1 equiv of TMP was used as base. [c] 1.3 ml of trifluoroethanol-chloroform was used as solvent.

[00248]   For the preparation of tripeptide XXVIII, HDATU was similar to or even slightly more effective than N-HATU.

[00249]   With respect to the test tripeptide XXX, the coupling of H-Phe-OBzl TosOH with Boc-Gly-Leu-OH in the presence of EDC/additive (coupling agent) in various solvents gave a product Boc-Gly-Leu-Phe-OBzl which was BOC-deblocked via 50% TFA/CH2Cl2 to give the crude tripeptide, which was directly analyzed by HPLC.

[00250]   In the EDC-mediated synthesis of XXX carried out in trifluoroethanol/chloroform (1:3 v/v), the three additives HODhat, HODhbt,

HOAt were found equally effective with less than 0.1% epimerization being observed. Upon switching to DMF as solvent, differences, although small, could be noted. Results are shown in Table 10.

[00251]   **Table 10** Effect of Coupling Reagent, Base and Solvent on the Preservation of Configuration during the Formation of *XXX* via [2+1] Coupling

Coupling Reagent	Solvent	Yield	LDL
EDC/HODhat	DMF	76.2	0.20
EDC/HODhbt	DMF	88.5	0.25
EDC/HOAt	DMF	90.6	0.33
EDC/HOBt	DMF	77.4	0.43
EDC/HODhat	TFE/TCM[b]	98.6	<0.1
EDC/HODhbt	TFE/TCM[b]	96.2	<0.1
EDC/HOAt	TFE/TCM[b]	98.2	<0.1
EDC/HOBt	TFE/TCM[b]	90.0	0.20

[b] Combination solvent trifluoroethanol-chloroform (1:3 v/v) was used.

[00252]   Following preliminary studies with simple di- and tripeptide models, XXVI, XXVII, XXVIII and XXX, a test peptide XXIX was assembled. The coupling of Z-Gly-Gly-Val-OH to H-Ala-Gly-Gly-OMe is a sensitive test for the nature of both coupling reagent and base. Results for the reaction in DMF, in the presence of collidine are gathered in Table 11. HDATU was found to be more effective in preventing loss of configuration at valine than N-HATU and other coupling reagents. Epimerization levels up to 8.2% of the DL-form were noted according to the order: HDATU<N-HATU<HDTU<N-HBTU.

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[00253]   Table 11 Effect of Coupling Reagent, Base and Solvent on the
Preservation of Configuration during the Formation of XXIX via [3+3] Coupling

Coupling Reagent	Base	Solvent	Yield (%)	LDL (%)
HDATU	TMP(3)	DMF	98.4	0.8
HDTU	TMP(3)	DMF	95.0	3.3
N-HATU	TMP(3)	DMF	96.6	2.4
N-HBTU	TMP(3)	DMF	85.6	8.2

EXAMPLE 26

[00254]   In order to demonstrate the suitability of the new HODhat-based
coupling reagent HDATU and compare its performance with that of the
corresponding guanidinium/uronium analogs N-HATU and HDTU in solid-phase
syntheses, 30 syntheses of the ACP segment H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-
Asn-GlyNH₂, (SEQ. ID. No. 2) were carried out by an Fmoc/*tert*-butyl protection
scheme as described in Example 22. Polyethylene glycol-polystyrene (PEGPS)-
resin bearing Fmoc-glycine was used as solid support. Peptide elongation was
performed manually, coupling times being shortened and excesses of reagents
being reduced in order to bring out the differences among the various coupling
reagents studied. Under these conditions, incomplete incorporations were
detected for Asn onto Gly, Ile onto Asn, Ile onto Asp, and Val onto Gln. Peptide
purity was judged by reverse-phase HPLC analysis, after cleavage from the resin
with TFA-H₂0 (9:1) for 2 hours at room temperature. The results are collected in
Table 12.

Table 12. Distribution of Products, Including Various Deletion Peptides, According to HPLC Analysis[a] for the Assembly of ACP via HODhat-HOAt- and HODhbt-Based Coupling Reagents[b]

Entry	Coupling Method	Solvent	Equiv.[c]	Preact. (min)	Coupling (min)	ACP (%)	-2Ile (%)	-Ile (%)	-Ile (%)	Val (%)	Asn (%)	Unkwn[d] (%)
1	HDATU	DCM	1.5	7	1.5	47	7	9	16	12	3	—
2	N-HATU	DCM	1.5	7	1.5	21	5	7	12	6	23	—
3	HDTU	DCM	1.5	7	1.5	4	13	5	13	2	3	—
4	HDATU	DCM	4	7	3	86	—	—	2	5	3	—
5	N-HATU	DCM	4	7	3	78	—	—	4	7	7	—
6	HDTU	DCM	4	7	3	31	5	7	26	3	4	—
7	HDATU	DCM	4	7	10	88	—	—	—	7	—	—
8	N-HATU	DCM	4	7	10	81	—	—	—	3	3	—
9	HDATU	DMF	1.5	0	1.5	68	3	10	17	2	1	—
10	N-HATU	DMF	1.5	0	1.5	87	—	3	1	2	7	—
11	HDTU	DMF	1.5	0	1.5	30	15	19	22	3	4	—
12	HDATU	DMF	2	0	5	97	—	1	—	2	—	—
13	N-HATU	DMF	2	0	5	94	—	1	1	2	2	—
14	HDTU	DMF	2	0	5	81	2	8	7	1	1	—
15	HDATU	DMF	1.5	7	1.5	60	1	7	7	1	5	16
16	N-HATU	DMF	1.5	7	1.5	77	—	7	—	3	11	7
17	HDTU	DMF	1.5	7	1.5	31	15	15	26	—	5	8
18	HDATU	DMF	4	7	1.5	72	—	5	3	—	18	2
19	HDTU	DMF	4	7	1.5	57	3	9	11	2	4	14
20	HDATU	DMF	3	7	3	64	—	1	1	—	4	20
21	HDATU	DMF	3	25	3	75	—	4	3	3	5	9
22	HDATU	DMF	4	7	30	95	4	1	—	2	1	—
23	N-HATU	DMF	4	7	30	86	2	—	2	1	1	—
24	HDTU	DMF	4	7	30	62	—	2	—	1	2	28
25	DIC-HODhat	DMF	4	7	30	55	9	—	—	12	—	—
26	DIC-HODhbt	DMF	4	7	30	29	—	—	—	—	30	5
27	Pfp[e]-HODhat	DMF	1.5[f]	7	1.5	5	—	—	—	—	3	—
28	Pfp[e]-HODhbt	DMF	1.5[g]	7	1.5	6	—	—	—	—	—	—
29	Pfp[e]-HODhat	DMF	3[f]	7	30	90	—	1	1	7	1	—
30	Pfp[e]-HODhbt	DMF	3[g]	7	30	87	—	3	3	6	1	—

[a] A reversed-phase C-18 column was used with elution by a linear gradient over 20 min of 0.1% TFA in MeCN and 0.1% aqueous TFA from 1:19 to 1:1, flow rate 0.1 ml/min. [b] In all HDATU- or HODhat-mediated syntheses, a bright-yellow-to-orange-red color change was observed within 2-5 min during amino acid coupling, except for Ile and Val. [c] Couplings were carried out in DMF or CH$_2$Cl$_2$ in the presence of 2 equivalents of DIEA per equivalent of Fmoc-amino acid/coupling reagent. [d] Extra peaks which appeared after 20 min on the HPLC traces. [e] Pentafluorophenyl ester. [f] Only 1 equiv of HODhat was used as catalyst. [g] Only 1 equiv of HODhbt was used as catalyst.

[00255]   Analysis of the chromatograms indicated that HDATU is far more effective than HDTU under all conditions examined, and more effective even than N-HATU in many instances. Methylene chloride was found to be a particularly suitable solvent for HDATU-mediated ACP synthesis. Thus, under so called "1.5 x 1.5" conditions in CH_2Cl_2, HDATU gave the decapeptide in a purity of 47%, whereas N-HATU and HDTU led to only 21% and 4% of the desired product, respectively. When a 4-equiv excess of reagents and a 3-min coupling time were used, 86% of acyl carrier protein (ACP) was obtained for HDATU, compared with 78% and 31% for N-HATU and HDTU, respectively.

[00256]   In DMF under "1.5 x 1.5" conditions, the performance of HDATU may not have been as efficient as N-HATU with or without preactivation. In addition, for preparation of model pentapeptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 (SEQ ID NO:3) which incorporates the highly hindered Aib-Aib unit, whether in DCM or DMF, HDATU was not able to equal the results obtained with N-HATU. For example, with 4 equivalents excess acid, 7 min preactivation and 30 min coupling time HDATU gave in DMF a peptide of 31% purity, whereas with N-HATU the purity was 91%.

[00257]   However, in general HDATU was found to be the better reagent under normal conditions. Thus, while using a 2-equiv excess of reagents without preactivation for a 5-min coupling, ACP was obtained in 97% purity by HDATU, whereas the corresponding values were 94 and 81% for N-HATU and HDTU. With 4-equiv/30 min coupling conditions with a 7-min preactivation time, excellent purity (95%) was obtained for HDATU, whereas with N-HATU and HDTU, the ACP purity was only 86 and 62%, respectively.

[00258]   When DIC/HODhat was used as a coupling reagent, satisfactory results were also obtained. Although not suitable under stringent conditions ("1.5 x 1.5"), HODhat could be used as an excellent catalyst and indicator in Fmoc-

amino acid pentafluorophenyl (Pfp) ester couplings under normal conditions. A bright-yellow-to-orange-red color change was noted which is much clearer than the color change from bright-yellow to pale-yellow observed with HODhbt. In DMF under conditions involving 3 equiv of pentafluorophenyl-ester and a 30-mm coupling time, both HODhat and HODhbt gave the desired ACP product in a purity of over 85%.

EXAMPLE 27

Fmoc-Ile-ODhat

[00259]   **Method A.** Under an atmosphere of dry N_2, a suspension of Fmoc-Ile-OH (0.3534 g, 1 mmol), HODhat (0.1805 g, 1.1 mmol) and $SOCl_2$ (0.73 ml, 10 mmol) in 8 ml of dry CH_2Cl_2 was refluxed overnight. Evaporation of CH_2Cl_2 and the excess of $SOCl_2$ gave a yellow solid, which was purified by flash chromatography through a short silica gel column with a mixture of EtOAc-CH_2Cl_2 (1:1 v/v) as eluent to give, after two recrystallizations from CH_2Cl_2-benzene-ether-hexane, 0.42 g (81%) of the analytically pure ester as a white solid: mp 160.5-162°C; ^{1}H NMR ($CDCl_3$): δ 9.15 (dd, 1), 8.58 (dd, 1), 7.96 (dd, 1), 7.76 (dd,2), 7.61 (dd, 2), 7.27-7.44 (m, 4), 5.20 (d, 1), 4.88 (q, 1), 4.49 (d, 2), 4.26 (t, 1), 2.21 (m, 1), 1.70 (m, 1), 1.34 (m, 1), 1.15 (d,3), 1.05 (t, 3); IR (KBr): 1811(s, COO), 1738(vs, CONN), 1692(vs, NHCO) cm^{-1}. Anal. Calcd for $C_{27}H_{25}N_5O_5$: C, 64.91; H, 5.04; N, 14.02. Found: C, 64.77; H, 5.23; N, 13.94.

[00260]   **Method B.** Under an atmosphere of dry N_2, 0.1854 g (0.5 mmol) of Fmoc-Ile-Cl was added with stirring to a solution of HODhat (0.0821 g, 0.5 mmol) and DIEA (95.8 µl, 0.55 mmol) in 10 ml of CH_2Cl_2 at 0°C. Stirring was continued at 0°C for 30 min and then at room temperature for 5 hours. The resulting light yellow solution was diluted to 30 ml with CH_2Cl_2 and washed quickly with ice-cold brine (2 x 15 ml). After drying over $MgSO_4$ and removing the solvent, the light yellow sticky solid was recrystallized twice from CH_2Cl_2-ether-hexane to give the analytically pure above-identified ester as a white solid: mp 161-162°C; NMR and IR spectra were identical with those of the sample obtained by Method A.

EXAMPLE 28

[00261]   Utilizing the procedure described in footnote a of Table 2 of the article by Carpino, et al., J. Org. Chem. 1995, 60, 3561, the contents of which are

incorporated by reference, the coupling of CBZ-Phe-Val-OH with H Pro-NH₂ to
form CBZ-Phe-Val-Pro-NH₂ was investigated using various coupling reagents.
Some of the coupling reagents used were those described elsewhere in the art
while others used were coupling agents of the present invention. More
specifically, for carbodiimide couplings, 0.105 mmol of Z-Phe-Val-OH, 0.1 mmol
of H-Pro-NH₂, and 0.11 mmol of the coupling reagent noted hereinbelow in the
table were dissolved in 1 mL of DMF or 1.3 mL of TFE/TCM (trifluoro
ethanol/chloroform) (1:3), and the solution was cooled in an ice bath and treated
with 0.11 mmol of EDC, EDC-HCl, or DCC. If a base is added, the number of
equivalents is given. The mixture was stirred at 0°C for 1 hour and at room
temperature overnight. The mixture was diluted with 25 mL of EtOAc and
extracted with 1 N NCl (2 x 5 mL), 1 N NaHCO₃ (2 x 5 mL), and saturated NaCl
(2 x 5 mL), dried with MgSO₄, the solvent was removed, and the crude peptide
was directly analyzed by HPLC. For onium salt couplings, 0.125 mmol of the
acid, 0.125 mmol of amide, and 0.25 mmol of base in 1 mL of DMF was treated
with 0.125 mmol of coupling reagent at 0°C and the reaction mixture was stirred
at 0°C for 1 hour and at room temperature for 2-3 hours, after which the workup
followed that described herein. In cases where an additive is used, one or more
equivalents of base (given in parentheses) may be added. The amount of loss of
configuration, as indicated by the presence of LDL epimer was determined. The
results are tabulated in Table 13.

[00262]   Table 13. [2+1] Segment coupling in solution (CBZ-Phe-Val-OH
+ H-Pro-NH₂).

Coupling reagent	Base	Solvent	% of LDL epimer
O-HATU	DCHMA[b] (2 eq)	DMF	7.91
t-Bu-Dtp-OAt	DIEA (2eq)	DMF	13.59
t-Bu-Dtp-OAt	DCHMA[b] (2 eq)	DMF	15.81
O-HATU	DIEA (2 eq)	DMF	10.07
Dtp-OAt	TMP (2eq)	DMF	1.77

Dtp-OAt	TMP (2eq) /HOAt (1 eq)	DMF	1.9
t-Bu-Dtp-OAt	**TMP (2eq)**	DMF	**1.63**
O-HATU	DB(DMAP) (2 eq)	DMF	1.59
O-HATU	Proton Sponge (2 eq)	DMF	2.78
Dtp-OAt	DIEA (2 eq)	DMF	16.04
O-HATU	DIEA/TMP (1/1 eq)	DMF	10.17

EXAMPLE 29

[00263]   Using the procedure as described in the article by Carpino, et al. in
Tetrahedron 1999, 55, 6813, the contents of which are incorporated by reference,
the coupling of Fmoc-Asp (t-Bu)-Phe-OH and F-moc-Lys (BOC)-PAL-PEG to
form Fmoc-Asp-(t-Bu)-Phe-Lys(-Boc)-PAL-PEG was conducted. Some of the
coupling reagents used were those described elsewhere while others used were
those of the present invention. Coupling reactions were carried out by deblocking
50 mg of H-Lys(Boc)-PAL-PEG-PS resin by means of 20% piperidine/DMF for 7
min, washing the resin with DMF, DCM and DMF (3 x 5 mL each) and then
adding a 5-fold excess (0.0475 mmol) of Fmoc-Asp(O-t-Bu)-Phe-OH (26.5 mg),
a 5-fold excess of the coupling reagent noted in Table 14 and 11.5 mg (0.095
mmol) of TMP or 12.3 mg of DIEA (10-fold excess) of the base, if any. In each
case the coupling reagent and the base were dissolved in 0.2 mL of the solvent
and the resulting solution added to the resin in a small syringe which served as the
reactor. Dissolution required about 1 min or less and care was taken to add the
solution as soon as possible after everything dissolved. This method is referred to
as the "low preactivation" method. Where preactivation was involved, the times
are recorded. The mixture was stirred gently every 10 min with a Teflon rod for
approximately 1 hour and then allowed to stand for 12 hours after which the resin
was washed with DMF and DCM (3 x 5 mL each) and deblocked by treatment
with 3 mL of TFA/H$_2$O (9:1) for 11/2 hour at room temperature. The solvent was
removed in vacuo and the residue dissolved in CH$_3$-CN for direct injection onto

an HPLC column under the following conditions: 4 μ 60A, C_{18} Waters Nova-pak
column, 3.9 x 150 mm; flow rate 1 mL/min; Waters 996 PDA detector; linear
gradient 10/30 in 20 min and then isocratic 30/70 for 20 min with
$CH_3CN/H_2O/0.1\%$ TFA; R_t (LLL-) 28.5 min, (LDL-) 30.5 min. The amount of
loss of configuration as indicated by the presence of LDL epimer was determined.
The results are tabulated in Table 14.

[00264]   Table 14 [2+1] Segment coupling under solid phase conditions[6]
(FmocAsp(tBu)-Phe-OH+ H-Lys(BOC)-PAL-PEG).

Coupling reagent	Base	Solvent	% of LDL epimer
N-HATU	TMP (2 eq)	DMF	18.88
N-HATU	TMP (2eq)	DCM	35.95
Dtp-OAt	TMP (2eq)	DCM	14.70
t-Bu-Dtp-OAt	TMP (2eq)	DCM	12.94
Dtp-OAt	TMP (2eq)	DMF	42.82

EXAMPLE 30

[00265]   Utilizing the procedure of Example 29, and utilizing CBZ-Gly-
Gly-Val-OH and H-Ala-Gly-Gly-PAL-Peg, CBZ-Gly-Gly-Val-Ala-Gly-Gly-
PAL-PEG (Sequence ID 4) was formed using t-Bu-DtP-OAt of the present
invention and O-HATU. The amount of loss of configuration was determined by
measuring the amount of LDL epimer formed. The results are indicated in Table
15 hereinbelow.

[00266]   Table 15. [3+3] Segment coupling under solid phase conditions
(CBZ-Gly-Gly-Val-OH + H-Ala-Gly-Gly-PAL-PEG).

Coupling reagent	Base	Solvent	% of LDL epimer
t-Bu-Dtp-OAt	TMP	DMF	1.99

| O-HATU | TMP | DMF | 2.09 |

[00267]   The high coupling efficiency of the coupling reagents of the present invention including t-Bu-Dtp-OAt was emphasized by solid phase synthesis of ACP decapeptide under the so-called "1.5x1.5" protocol. Under these demanding conditions the coupling efficiency of various coupling reagents can be easily brought out. The couplings are carried out for 1.5 minutes using a 1.5-eq excess of protected amino acids and 1.5 eq of coupling reagent in the presence of 3 eq of base.

[00268]   For manual solid phase syntheses of ACP under the 1.5x1.5 protocol, using the procedure of Carpino, et al., in J. Chem. Soc. Chem. Comm, 1994, 201, the contents of which are incorporated by reference and using O-HATU, Dtp-OAt and t-Bu-Dtp-OAt the purity of the crude peptide was 76 %, 60 % and 74 %, respectively. The new phosphorus-based coupling reagent therefore at least equals the effectiveness of O-HATU, considered the best of the previously described reagents.

[00269]   The above preferred embodiments and examples are given to illustrate the scope and spirit of the present invention. These embodiments and examples will make apparent to those skilled in the art other embodiments and examples. These other embodiments are within the contemplation of the present invention. Therefore, the present invention should be limited only by the appended claims.